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Contents

Pages

1. Agriculture
   Absts. No. 1-9 1

2. Agricultural Chemistry
   Absts. No. 10-93 5

3. Agricultural Economics
   Absts. No. 94-95 45

4. Agricultural Engineering
   Absts. No. 96-108 49

5. Fisheries
   Absts. No. 109-137 58

6. Forestry
   Absts. No. 138-189 72

7. Zootechnical and Veterinary Science
   Absts. No. 190-220 95

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I. Agriculture

Abstract Nos. 1—9

1. **Some problems on the agriculture of highland in Japan.** Yakichi NOGUCHI (133 p., 19 illus. Nôrintôkei-kyôkai, Tokyo, 1952)

The study carried out on the practice of agriculture which was found in highland in the Middle Japan to get some informations about the effective method of reclamation in highland. The highland in the Middle Japan has very short non-frost season. It receives, however, abundant sunshine in summer even though cool air, so that the difference of temperature between day and night is generally large. The amount of snow and rainfall increase by degrees with the elevation. In winter, winds blow very strongly, therefore it is necessary to protect agriculture against winds. Soil erosion is very often found on the slope of mountain skirt.

Paddy fields spread out mostly at the elevation of 1000 M above sea level, but to select the early-ripened varieties and to take special care of growing seedlings are very important points of rice culture in the highland. Ryes, oats, potatoes and several kinds of vegetables, for instance, cabbages and lettuce, have fine crops there, and yet the proper rotation system and the combination of animal husbandry are two keys to succeed the crop production. In general, the agriculture is very small scale and the management is almost self-support.

The records about some instances of reclamation which have advanced after the war are also given in this paper.

2. **On the absorption and assimilation of foliar-applied urea.** Yakichi NOGUCHI and Tomota SUGAWARA (Science [Japan] **22**, 386-390, 1952)

Over an half amount of 0.1 *N* water solution of urea foliar-applied was let into the leaves of tobacco plant during 24 hours and the green color of the leaves changed darker gradually caused by the increase of chlorophyll and other chromatic substances. The fact proved that absorption of urea by leaves was fairly rapid. It may be said that urea permeated into leaves though cuticle, because the absorption occurred even on the surface of leaves, on which on stoma was found. The contents of nitrogen compounds and amino acids in the leaves of tobacco and rice plants were examined after spraying urea and the tendency of increasing was seen in all kinds of nitrogen compounds, especially free amino acids. So it may be decided that the assimilation process of nitrogen which came into leaves with spray of urea was just like that of nitrogen absorbed from soil with roots. Remarkable injury appeared so often on the surface of leaves after urea spraying when the plant was kept in dark, and the defect was recovered with the spray of cane sugar solution on the leaves, so that carbohydrate was supposed to be the supplier of energy for the assimilation of nitrogen in leaves.

3. **Studies on the formation of ascorbic acid (vitamin C) in plants. 5. The germination of seeds and the production of ascorbic acid.** Tomota SUGAWARA (Jap. Journ. Bot. **14**, 125-46, 1953)

As experimental materials the seeds of twenty species were used, and they

were treated with 0.1 per cent mercuric chloride solution for 30 minutes and then thoroughly washed. Seeds were germinated on moist quartz sand or filter paper in dark thermostat kept at 25° or 30°C. At certain definite periods in samples were collected and the ascorbic acid was determined according to the FUJITA's method.

The results obtained in the present study show that ascorbic acid is produced by germinating seed in a considerable amount. It depends, however, a great deal upon the character and kind of seed and upon the external or internal factors such as temperature, light, water, oxygen, sugar content, enzyme activity, and amount of micro elements in seeds. On the other hand, formation of ascorbic acid during germination is also affected by the physical and chemical treatments of seeds such as X-ray, ultra-violet rays, synthetic growth substances, vitamin B_1, ascorbic acid, and micro elements (manganese and boron).

Light did not affect the formation of ascorbic acid at the earliest stage of germination, when plentiful storage of nutritive substance is available, but a marked effect was found at later stage of germination, when storage had been almost exhausted. The amount of ascorbic acid in germinating seeds after 120 hours illumination was, in average, about 65 per cent greater than in that under the dark condition. The oxygen is essential for the formation of ascorbic acid as such. In anaerobically cultivated rice seedlings, the elongation of coleoptils was observed, but no increase in the total amount of ascorbic acid took place. Synthesis of ascorbic acid may be affected by means of a supply of manganese and boron from outside. The amount of ascorbic acid increased when seeds germinated in the dark and were supplied with micro elements. But there was no direct relation between the amount of manganese contents in seeds and the formation of ascorbic acid during the seed germination. The close correlation was confirmed between the production of ascorbic acid and peroxidase activity in plants. Such tendency, however, was not found in the activity of ascorbic acid oxidase.

It is evident that germination of seeds and growth of seedlings under suitable conditions accompany formation of more ascorbic acid. The formation of ascorbic acid can not be regarded as, however, essential for the growth of plant; the rate of the formation of ascorbic acid fell and became nearly zero after about 10-12 days of germination in the dark, but the rate of growth continued unchanged, and in consequence the concentration of ascorbic acid steadily fell. In many plants ascorbic acid is produced in abundance and the growth of them is not affected by the quantity of the substance, but only under exceptional conditions, which bring about its decrease, the growth is suppressed.

4. Some observations on the consumption of oxygen by rice roots grown in water cultures. Kanoe SATO (Proc. Crop Sci. Soc. Japan **21**, 16-17, 1952)

The author studied the effects of submergence on the growth of rice plants, and he must know the aeration of soil and the respiration of rice roots under submergence. The oxygen consumption of rice roots with its development of growth in water culture—the renewal of the solution was generally practised at intervals of 2 days—was measured by the WINKLER's method: the quantity of oxygen consumed each day was determined by the decrement of oxygen in the solution during 4 hours from 10 a.m. to 2 p.m.

(1) The oxygen consumption increased rapidly since the beginning of panicle

differentiation or culm elongation, attaining its maximum at the beginning of heading, and decreased gradually thereafter.

(2) The rate of respiration of rice roots varied in parallel with the temperature of the solution; even at the booting or heading stage it dropped suddenly at lower temperatures.

(3) As almost all the oxygen in the cultural solution was exhausted during a daytime or a day, the theory that the oxygen transports from the aerial parts of the plant to the roots might be supported.

5. On the elongation of the leaves and internodes of the main-stem in rice plants. Kanoe SATO (Proc. Crop. Sci. Soc. Japan **21**, 75-76, 1952)

(1) The lengths of all leaf-blades and -sheaths and internodes of the main-stem, were measured on 9 rice plants sampled every other day since transplanting, intending to know the elongation process of each leaf and internode and further to make clear the interrelations between them.

(2) The elongation of each leaf and internode was very slow during the time between the differentiation and its reaching 1.0 cm and 3-5 mm in length respectively, taking about half a month by that time, and another half a month thereafter till elongation ceased.

(3) The elongation curves of leaves and internodes were so-called "S-curves", the former being composed of 2 S-curves which represented the elongation of blade and sheath of a leaf respectively.

(4) When the blade of the 10th leaf, for instance, began its rapid growth the 9th leaf-blade and the 8th leaf-sheath projected together—always the former was a little longer—above the top of the 7th leaf-sheath. When the blade of the 10th leaf attained its full size, its sheath still continuing to grow, the internode below its base might be 2 or 3 mm long. All internodes, which were due to grow long, did so after the leaves borne at their respective upper ends had ceased to do so.

(5) As the locus went up, the leaves beared blades successively larger and reached the maximum in the 3rd (in var. North No. 17) or 4th (in Norin No. 8) leaf from the boot leaf, the blade of which was shorter and broader than that of its predecessors.

6. Magnesium deficiency in grapes. Tadayoshi SUGIYAMA, Masatoshi IWATA and Hitoo YASHIRO (Journ. Hort. Ass. Japan **21**, 161-164, 1952)

In the summer of 1951, the authors surveyed the vineyards in Yamanashi Prefecture, and found a chlorotic symptom was widely distributed there. The symptom had developed on the basal leaves in July or later on, while apical leaves remained healthy. In the affected leaves, interveinal portions became chlorotic and later turned brown in severe cases. Premature defoliation occurred in those vines.

Leaf and soil samples were collected and analyzed. Magnesium contents were very low in the chlorotic leaves, usually less than 0.25% on dry matter basis, while they were high in the healthy ones. The symptom and the analytical results seemed to show that the chlorosis found on the grapevines was due to magnesium deficiency. The soils of the chlorotic vineyards were not always low in replaceable magnesium, but were sometimes high in potassium or calcium. It was suggested that antagonistic relation between the bases played a role in the occurrence of magnesium deficiency in the grapevines.

7. Genecological studies on cultivated rice. Takane MATSUO (Bull. Nat. Inst. Agr. Sci. Series D. No. 3, 1952)

In an attempt at classifying several thousand varieties of rice cultivated throughout the world from a genecological standpoint, we investigated their morphological and ecological characters, with the following results:

(1) We classified them into 43 plant types on the basis of 22 external morphological characters such as grain type etc., and further divided them into the 3 main plant types of A, B and C. From the relationships between them and geographical distribution of the varieties, we recognized that varieties that have their origin and spread in common and are genetically akin show analogous forms, and that identical environments tend to bring about analogous morphological types even of rices of different strains.

(2) Some representatives of these varieties were also classified by the differences on damages by rice-borer, in degree of affection by rice-blast, in tillering and lodging and other physiological characters by fertilizing, in premature heading of seedlings and in days from seedling to heading by the controlling of the day-length and temperature.

(3) When the meteorological ecotype, soil ecotype and biological ecotype are determined according to the growth reaction respectively to meteorological, soil and biological environments as the results of above tests, it is recognized that it is very closely related with the classification by plant type and geographical distribution, and that the selection of ecological characters by environments is effected in parallel with the morphological characters by environments.

(4) When we observe the differentiation of the cultivated rice and origin of the Japanese rice on the basis of the above mentioned classification, it is thought that rices of the 3 plant types A, B and C, were the wild rice (*O. sativa* L. f. *spontanea*) under cultivation independently in South-China, Java and near India, or that the B type were differentiated in Java and China respectively from the Indian C type as the origin type. The Japanese rice is probably the A type in middle China brought to northern Kyushu and differentiated.

8. Studies on the mechanism of the expressions of the translucent and lemon genes in the silkworm. I. On the expressions of the translucent and lemon genes in the silkworm eggs. Hisao ARUGA, Narumi YOSHITAKE and Masaharu EGUCHI (Journ. Seric. Sci. Japan **21**, 255-263, 1952)

The investigations on riboflavine, 3-hydroxykynurenine, kynurenine and two pterin compounds have been carried out by the methods of paper chromatography and diazo reaction using *od* translucent, lemon, white-1, white-2 and normal in order to determine the mechanism of the expressions of *od* translucent and lemon genes. The serosa cell pigments in *od* translucent egg show lighter color than those of normal, and yet in the case of hybrid with normal this phenomenon is observable only when the *od* translucent female moth is used. It may be plausibly thought that three main factors have closely relations with each other for the above mentioned phenomena: (1) the qualitative difference of an unknown chemical substance S_1 between normal and *od* transmitted from female moth to egg, (2) the difference of the expression of *od* gene controlled by S_1 substance, (3) the difference of the expression of $+^{w_2}$ gene whose action is inhibited by the S_2 cytoplasmic condition

influenced by *od* gene.

Lemon is characterized by the yellow skin color of the larva due to the existence of xanthopterin-B, which is not only transmitted from mother moth to the egg, but also synthesized in the embryo by the action of *lem* gene from unknown chemical substance, probably from its precursor.

The quantity of riboflavine present in *od* translucent egg is less than normal, and in general riboflavine tends to fairly increase during the embryonic development.

9. Studies on the mechanism of the expressions of the translucent and lemon genes in the silkworm. II. On some biochemical substances in the silkworm larva. Hisao ARUGA, Narumi YOSHITAKE and Ren ISHIHARA (Journ. Seric. Sci. Japan **22**, 1-8, 1953)

It has been observed that the larvae such as Striped (p^S), Moricaud (p^M) and Ursa pattern (U) containing much melanin pigments in the primary cuticle contain more leucopterin-B and less uric acid in epidermis than normal ($+^p$), and those of p^S *lem*, p^M *lem* and U *lem* contain less xanthopterin-B in their epidermal cells than normal lemon.

Xanthopterin-B is hardly be formed in the epidermal cells of a few translucent and marking larvae containing small quantities of uric acid in epidermis.

From above mentioned phenomena it may be plausibly considered that xanthopterin-B and uric acid can not be accumulated or formed in the epidermal cells resulting from changes of some cytoplasmic conditions accompanying with melanin formation. The melanin formation from dopa is inhibited by xanthopterin-B and accelerated by leucopterin-B *in vitro*, in spite of the fact that such phenomenon is not recognizable *in vivo*.

From the results of the investigations as to the changes of uric acid, pterins, kynurenine and 3-hydroxykynurenine in the adipose tissue from the stages of larva to pupa, it has been recognized that some parts of these substances moved from the epidermal cells or blood to the adipose tissues accompanying with metamorphosis, and these changes of physiological functions are in accordance with the morphological changes of the adipose tissue.

II. Agricultural Chemistry

Abstract Nos. 10—93

10. Dynamic studies on the nutrients uptake by crop plants. (Part 4). The mechanism of nitrite formation by metabolising plant roots. Shingo MITSUI and Kiyoshi TENSHO (Journ. Sci. Soil and Manure, Japan **23**, 5, 1952)

A series of water culture study elucidated the mechanism of nitrite formation in the rhyzosphere of metabolising plant roots as follows:

(1) The nitrite formation is a reduction process from nitrate. No sign of oxidation from ammonium nitrogen was observed.

(2) The redoxpotential Eh_6 of the water culture solution lowered from 0.65-

0.68 volt to ca. 0.55 volt on the abundant accumulation of nitrite nitrogen. As soon as hydrogen sulfide evolved it lowered even to ca. 0.14 volt. The fact indicates that nitrite formation is a reliable sign of mild reducing power of metabolising roots.

(3) Bubbling ordinary air through the medium tremendously inhibited nitrite formation.

(4) The nitrite formation was also remarkably inhibited by either darkening or cutting off stems and leaves with simultaneous decrease of organic reduceable matter (Permanganate titration) in the medium.

(5) Although addition of glucose, asparagin and citric acid as energy materials to non-sterile culture medium lacking metabolising roots promoted nitrite formation to some extent, it was far less and slower as compared with its usual accumulation by metabolising roots.

All the experiments mentioned above may lead to the definite conclusion that the simultaneous and cooperative effect of the downward supply of some energy materials depending on the normally metabolising upper parts of the plant (Photosynthesis) and of the depression of oxygen partial pressure in the medium by metabolising (aerobic respiration) plant roots should contribute to the nitrite formation in the rhyzosphere.

11. Dynamic studies on the nutrients uptake by crop plants. Part 5. On the determination of radioactive phosphorus (P^{32}) in the plant nutritional studies. Shingo MITSUI and Kikuo KUMAZAWA (Journ. Sci. Soil and Manure, Japan **23**, 131, 1953)

Special precautions should be given to the handling of ultra-dilute solution of $P^{32}O_4$ and the determination of its intensity by plant ash as such.

The absorption error of $P^{32}O_4$ on the surface of glass-ware sometimes oversteps the allowable limit of experimentation, when its contact proceeds at such ultra-dilution as is often the case in diluting $H_3P^{32}O_4$ sample of high specific activity. Practically, 0.5 ppm addition of inert PO_4 as carrier proved sufficient to minimise this reduction to negligible extent.

The absorption effect of plant ash in the determination of $P^{32}\beta$-ray by GEIGER-MULLER Counter was studied. Despite of the powerful maximum energy of β-ray emitted from P^{32} (1.67 MeV.) self-absorption of plant ash containing the same specific activity of P^{32} reached ca. 3% and 5% at 20 mg and 30 mg ash per square centimeters respectively.

12. Dynamic studies on the nutrients uptake by crop plants. Part 6. Studies on the exchange depletion of Ca^{45} from plant roots. Shingo MITSUI and Tomoyoshi KIUCHI (Journ. Sci. Soil and Manure, Japan **23**, 178, 1953)

The depletion of Ca from metabolising barley and wheat roots was studied by employing tracer technique of Ca^{45}.

The outward movement of Ca^{45} was remarkable in the chloride of K, NH_4, Rb, Mg, Ca, Sr and Ba except for Li and Na in contrast to H_2O. The highly hydrated ions as Li and Na might have failed to deplete Ca. Unexpectedly, however, no significant differences were observed among other ions. This is the marked contrast to K^{42}, which has been reported to be exclusively depleted by K.

A series of enzymatic inhibitors such as NaCN, NaN_3, NaF, Na-Iodoacetate,

Na-Malonate and Dinitrophenol did not affect the depletion except for dinitrophenol. The elevation of temperature from 6°-20°C, however, more or less promoted its outward movement. This apparent discrepancy was discussed on the basis of metabolic activity. Further, different anions showed no remarkable difference.

The alternate treatment with H_2O and salt solutions for the same plant root gave definite proof that the depletion, so far as this study was concerned, was a normal process of healthy metabolising roots and no sign of injury, as might be expected on the use of single salt solution, could be observed.

In conclusion, the status of Ca held in metabolising plant roots seems to be more or less loose as compared with K and yet a little stronger than Na, its linkage being almost, if not entirely, independent of the normal metabolism.

13. Studies on the amino acids and amides metabolism of rice plant. Part 2. Kiyoshi OZAKI and Masaaki MORIYAMA (Journ. Sci. Soil and Manure, Japan **23**, 9, 1952)

Following the previous report, the authors performed an experiment as the first step to study the metabolism of amino acids and amides in rice plant.

The authors examined with paper chromatography the free amino acids and those contained in protein hydrolyzates in various organs through the whole growth period with various nitrogen sources.

The following results were obtained.

(1) The free amino acids in the tissue of the plant were found to be almost always aspartic acid, glutamic acid, serine (or including glycine), alanine. Therefore, it was supposed that these were the main materials for the metabolism of amino acids. In addition to these amino acids there were often found valine and lucine. Sometimes, threonine, tyrosine, β-alanine, γ-aminobutyric acid and basic amino acids were also found.

(2) Free proline was found only in pollen. The special function of proline was postulated in this organ.

(3) Almost all sorts of ordinary amino acids were found in protein hydrolyzates.

14. Increase of protein content in white rice by the nitrogen application at shooting. Kiyoshi OZAKI and Masaaki MORIYAMA (Journ. Sci. Soil and Manure, Japan **23**, 146, 1953)

White cleaned rice generally is the staple food in Japan constituting the principal carbohydrate source. The shortage of the protein content of it, however, has caused the main deficiency of the Japanese in nutrition.

In a series of nitrogen fertilisation study of rice plant, we found that the quantity of protein in rice was increased even by more than ten per cent by the nitrogen application at shooting time although the grain yield was not altered.

The effect of this nitrogen application was mainly on the increase of oryzanine, which is the main stored protein in white rice, and slightly on the increase of rice bran nitrogen, the thickness of aleuron layer being unaltered.

Unexpectedly, no significant improvement was observed in the quality of rice protein; the outstandingly low percentage of lysine and threonine was indifferent to the treatment.

15. Studies on dextran. III. Periodate oxidation of dextran. Tsuneo

Kobayashi and Yutaka Tsukano (Journ. Agr. Chem. Soc. Japan **27**, 33-7, 1953)

The periodate oxidation method was applied to the oxidation of dextrans from various strains of *Leuconostoc mesenteroides* and unidentified new strains of dextran-forming bacteria. The ratios of 1,4- and 1,6-glucosidic linkages in these dextrans were calculated by measuring the amount of formic acid produced and it was found that they were varied from 1 to 5 to 1 to 16.

The reaction velocity was about the same in all cases, and no over-oxidation was observed. The oxidation product was isolated, and its properties were described. The stability of formic acid produced was discussed and confirmed.

16. Limit dextrinase activity of molds. Tsuneo Kobayashi and Kunio Yamanouchi (Journ. Agr. Chem. Soc. Japan **27**, 180-6, 1953)

The amylolytic enzymes of *Aspergillus oryzae* and *Aspergillus usamii* were fractionated by repeated salting-out with ammonium sulfate, precipitation with acetone, and specific precipitation of α-amylase with rivanol. Variations in the activities of dextrinogenic and saccharogenic amylases, limit dextrinase, and maltase were followed during the fractionation procedure, special attention being paid on the relation of limit dextrinase activity (LDA) and other enzyme activities. α-Amylase activity and LDA could be easily separated by various treatments. But the latter was always accompanied with saccharogenic activity. No enzyme fraction was obtained, which showed only LDA. α-Amylase was purified to be almost free from LDA, but even the crystalline α-amylase showed very low LDA.

The limit of hydrolysis by LDA was estimated on a malt limit dextrin of the mean polymerization edgree of 7.3. This dextrin was split to about 85% by prolonged action of the enzyme, leaving a residue which had a polymerization degree of 2. The fact would suggest that 1,6-glucosidic linkages were resistant to LDA.

The optimum conditions for LDA and saccharogenic amylase activity were about the same, i.e., at pH 4.8 and 65°C, while those of α-amylase were different, i.e., at pH 5.6 and 70°C. α-Amylase was more sensitive than the other activities to the inhibition by mercuric chloride.

Following conclusion was given on the basis of foregoing results: The LDA in mold enzyme might not be ascribed to the presence of an independent enzyme, limit dextrinase, but rather to the auxiliary action of ordinary amylase, among which the effect of sacchrogenic amylase predominated.

17. Studies on chemical reactions with high frequency oscillator. III. A method to measure the reaction of urease with a high frequency titration apparatus. Tetsuo Kôno (Journ. Agr. Chem. Soc. Japan **26**, 533-6, 1952)

(1) A high frequency titration apparatus was used to study the action of urease upon urea, and the reaction processes were plotted precisely from the very beginning of the reaction.

(2) The facts formerly known, the activation of a crude urease by glycine, and the inactivation by means of Ag^+ or diazobenzenesulphonic acid, were confirmed.

(3) When titrated with 0.01 N HCl or H_2SO_4, the enzymatic digest gave a three-stepped curve. A solution of $(NH_4)_2CO_3$ and enzyme gave the same one, but that of $(NH_4)HCO_3$ and enzyme gave a two-stepped one.

(4) The method was examined quantitatively, and was found to be almost

satisfactory. It can be used, of course, for the quantitative analysis of urea, with the help of urease.

18. The preparation of reagents for micro analysis of carbon and hydrogen. Tetsuo KÔNO, Setsuko YAMAMOTO and Michiko NAKAYAMA (Japan Analyst. **1**, 126-30, 1952)

Conditions to prepare the first grade reagents (CuO, $PbCrO_4$, PbO_2, and asbestos) for micro analysis of carbon and hydrogen were examined. A high frequency electric conductometer was designed and used to watch the rinsing process of the reagents. The reagents thus obtained gave a satisfactory results on analyses after only 1 or 2 hour preheating.

19. Notes on organic micro analysis. Tetsuo KÔNO, Setsuko SHIMOKÔBE, Michiko NAKAYAMA and Mitsuko OGATA (Ibid. **2**, 137-41, 1953)

Modifications for organic micro analyses were proposed.

(1) A micro-weighing tube which can be screwed to a glass stick was proposed for the sampling of micro CARIUS method.

(2) Modification of nitrometer for micro DUMAS method. The cock at the top of the meter was modified and a Pt wire was sealed at the bottom of it. The wire was used to oxidize electrically the surface of the mercury so as to prevent the attaching of CO_2 bubbles on it. The oxidation was done with 3 v. battery, of which + pole was connected to the Pt wire, while — pole was inserted to the KOH solution in the leveling bulb.

(3) A convenient electric furnace for micro carbon and hydrogen analyses was described.

(4) KÜSTER'S table for the calculation of micro-DUMAS method was recalculated by adding the correction of -2%.

20. Oxidative phosphorylasion in higher plant homogenate. Bunji MARUO and Michio MATSUHASHI (Journ. Agr. Chem. Soc. Japan **26**, 440, 1952; **27**, 215-18, 1953)

A direct decrease in inorganic phosphate under aerobic conditions was observed in a homogenate system from the higher plant, as it has been found in animal homogenate.

Phaseolus mungo seedlings were homogenated in a solution which consisted of 0.087 M succinate, 0.0013 M ATP, 0.013 M phosphate, 0.013 M $MgSO_4$, 1.3% glucose, 1.3 mg./cc. hexokinase, and 0.06 M NaF. The homogenate obtained was filtered through gauze and shaken in a Warburg apparatus at 37°. A remarkable decrease in inorganic phosphate was observed for 10-30 minutes and the P/O ratio obtained was about 0.33.

21. Mechanism of formation of starch in the leaves of higher plants. II. Bunji MARUO, Takashi AKAZAWA and Yasuji IIMURA (Journ. Agr. Chem. Soc. Japan **27**, 207-9, 1953)

The starch formation in leaf was studied using leaf discs floating on various substrate solutions under various conditions. The necessity of oxygen for the starch formation in leaf discs from glucose was observed. Furthermore, it was found that arsenate or 2,4-dinitrophenol inhibited the synthesis completely, without any marked

effect on respiration. These data, together with the previous report(1), seem to suggest the possibility that in higher plants too, oxidative phosphorylation should play an essential role for energy translation.

22. Inhibitor of oxidative phosphorylation which is contained in plants tissues. Bunji MARUO, Yasuji IIMURA and Michio MATSUHASHI (Journ. Agr. Chem. Soc. Japan **27**, 210-13, 1953)

It was found that extracts of higher plant tissues inhibit the oxidative phosphorylation in an animal tissue homogenate system. The inhibitor was identified as Ca^{++}. The inhibiting action of Ca^{++} was eliminated most effectively by the previous addition of NaF.

23. Effect of light on the phosphorus metabolism of Chlorella. Bunji MARUO, Michnori NAKAMURA, Takao ÔMURA, Tetsuo KÔNO and Shirô MATSUMOTO (Journ. Agr. Chem. Soc. Japan **27**, 348-53, 1953)

The phosphate metabolism of *Chlorella ellipsoides* was investigated under several conditions using $H_3P^{32}O_4$ as a tracer. In the light, the activity added to the external medium was incorporated into the *Chlorella* cells much more quickly than in the dark. Presence of CO_2 and/or O_2 showed little effects. The incorporation in the light was inhibited by NaN_3 or KCN. The *Chlorella* cells treated in various conditions were harvested and the distribution of the amount of phosphate and of the activity was compared. Any remarkable change caused by presence and absence of light, CO_2, or $CO_2 + O_2$ was not detected.

24. Studies on the enzymic formation and degradation of starch. XIV. Potato phosphorylase. 1. Michinori NAKAMURA (Journ. Agr. Chem. Soc. Japan **26**, 260-7, 1952)

Potato phosphorylase was purified by the ammonium sulfate precipitation method, accompanied by heat treatment at 50° for 5 min. and dialysis against running water. The purest preparation showed the activity of 2.78 units/mg. protein (at 38°), which was approximately in the same order with the purest preparations of the former investigators. The synthesis of starch from glucose-1-phosphate, as catalyzed by this enzyme, proceeded according to zero order reaction rate in its early stage until the value, *Pinorg.* / (*Pinorg.* + *Pester*), reached 0.2. The optimum pH of this reaction was at pH 5.9 (5.5-6.3). The dissociation constant between the enzyme and glucose-1-phosphate (Michaelis' constant), as determined by the graphical method, was $1.5 \times 10^{-3}M$ glucose-l-phosphate. The amount of activator polysacharide which gave half maximal velocity was 41.1 mg. soluble starch per 100 ml. The effects of various carbohydrate activators on the purified enzyme were examined to give the results essentially similar to those by earlier investigators. The amylose-type polysaccharide, produced by the action of isoamylase on glutinous rice starch, was 80% as active as the soluble starch. Dextran and its hydrolysis products were entirely inactive. Temperature coefficient of this reaction between 17-42° was approximately 2, with the apparent activation energy of 12.6 kcal./mol. Energy, heat content, free energy, and entropy of activation of the denaturation of this enzyme by heat

(1) B. MARUO and M. NAKAMURA: Journ. Agr. Chem. Soc. Japan **25**, 245, 1951.

(37-47°) were determined by using the theory of rate processes of Eyring with results of 49.4 kcal./mol., 48.8 kcal./mol., 24.0 kcal./mol., and 78 cal./deg. mol., respectively; these results were in support of the protein nature of the enzyme. Purified potato phosphorylase did not require the presence of adenylic acid, cysteine, and Mg for full activity. Equilibrium of the reaction, as catalyzed by purified enzyme at pH 6.6-6.8, was established when the value, $P_i/(P_i\ P_e)$, reached 0.80-0.84; from this value, the equilibrium constant of this reaction was calculated as 4.0-5.3. The free energy change of this synthetic reaction was, therefore, approximately —1.0 kcal./mol. (pH 6.6-6.8).

XV. **Potato phosphorylase. 2.** Michinori NAKAMURA (*Ibid.* **26**, 267-72, 1952)

The effects of various inhibitors on the synthesis of starch from glucose-l-phosphate, catalyzed by purified potato phosphorylase (18.7 units/ml. and 2.12 units/mg. protein at 37°), were tested and the results were compared with those by previous workers. Heavy metals, such as Ag^+, Cu^{++}, Zn^{++}, and Hg^{++}, had markedly inhibitory effects in very low concentrations, while Pb^{++} was hardly effective in much higher concentrations. Phytohormones, such as α-naphthylacetate and 2,4-dichlorophenoxyacetate, and adrenalin had more or less inhibitory effects on this enzyme. Insulin, on the contrary, had an activating effect, antagonizing with the inhibitory effect of adrenalin. Arsenate inhibited the phosphorolysis reaction of starch by competing with the enzyme with inorganic phosphate. The degree of inhibition by arsenate was nearly directly proportional to the molar ratio of As to P in the reaction mixture.

XVI. **Failure of cyanide to inhibit β-amylase.** Michinori NAKAMURA (Journ. Agr. Chem. Soc. Japan **26**, 497-502, 1952; Science **117**, 629-31, 1953)

Treatment of malt extracts with 0.1% KCN at 37° for 1 hr. had no effect on the α-amylase activities, but lowered the saccharogenic activities of these solutions. This treatment had marked decreasing effect on the α-amylase activities of Takadiastase extracts, and on the β-analyse activities of extracts from ungerminated wheat, barley, soybean, and sweet potato, and of solutions of purified β-amylase from sweet potato. This decreasing effect was dependent on the source of the enzyme as well as on the enzyme concentration in the treating mixture. Treatment of these enzyme solutions with 0.1% HCN at pH 5.0 had no decreasing effect on both α- and β-amylase activities. Potassium carbonate, in 0.1% concentration, showed approximately the same degree of inhibition on these enzyme solutions as compared with 0.1% KCN. Moreover, these 2 reagents had approximately the same raising effect on the pH values of the treating mixtures. The above results support the view, in contrast to that of Roy and Underkoffler (cf. *Cereal Chem.* **27**, 404, 1950), that cyanide has no inhibitory effect on both α- and β-(or saccharogenic) amylase activities of the enzyme solutions used in this investigation. The apparent inhibitory effect of NaCN, which they observed in their experiments, may be results of combined effects of pH and enzyme concentration on the pH-heat-stability properties of enzyme solutions.

XVII. **Effect of added primer on lima been phosphorylase.** Michinori NAKAMURA (Journ. Agr. Chem. Soc. Japan **27**, 70-5; Nature **171**, 795-6, 1953)

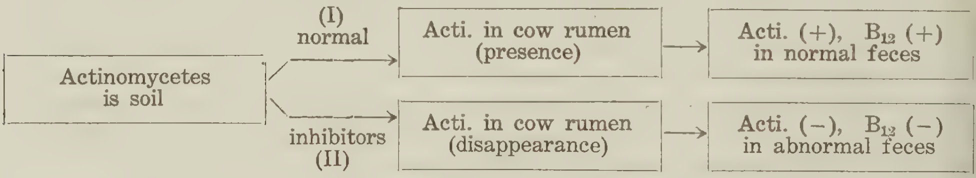
In the synthesis of amylose-type polysaccharide from glucose-l-phosphate by the action of phosphorylase, it requires the collaboration of the pre-existing polysac-

charide—usually called as primer or activator—for the priming of the reaction. However, according to GREEN and STUMPF (cf. *J. Biol. Chem.* **142**, 355, 1942), it was reported that purified lima bean phosphorylase required no added primer in the synthesis of starch. To study further on this question, lima bean phosphorylase was purified according to GREEN and STUMPF. The purity of the enzyme solution thus far obtained was much lower than that of purified potato phosphorylase. Nevertheless, this preparation required added primer in the synthesis of starch, and the Michaelis' constant, as determined by the graphic method, was approximately 30 mg. Lintner's soluble starch per 100 ml. The purest preparation still contained a trace of polysaccharide, which could serve as a primer source for purified potato phosphorylase, and the priming activity of which disappeared on treatment with β-amylase. The amount of polysaccharide in this preparation was not determined, but it was found by comparing the priming power of this polysaccharide with that of soluble starch that the amount was far less than the amount which was sufficient to give an error of 5% in the determination of the Michaelis' constant.

25. Biosynthesis of vitamin B_{12} in various organisms. I. Yoshikazu SAHASHI, Kiichi IWAMOTO, Masayuki MIKATA, Akihiko NAKAYAMA, Heiichi SAKAI, Jusaku TAKAHASHI, Junzo HAYASHI, Nobuo SENO, Tadami AKATSUKA, Taihei MIKI, Keiji HARASHIMA and Rentaro MATSUMOTO (Journ. Biochem. Japan **40**, 227-244 1953; Proc. Japan Acad. **28**, 573-577, 578-580, 581-584, 1952)

Recent studies on the biochemistry of vitamin B_{12} are worthy of deep interest and numerous papers have been published, but the mechanism of biosynthesis of this vitamin has not been studied so far. The present paper is stated concerning these problems in various organisms in Japan.

(1) The normal biosynthesis in vivo of vitamin B_{12} in cattle seems to take place according to the shema (I) and abnormal metabolism the shema (II).



Considerable kinds of elements or compounds such as SiF_6'' ions in nature may be physiological inhibitors for vitamin B_{12}-production in various organisms as in the case of "Kuwazu disease".

(2) No anemia was recognized in rats in any case of the vitamin D-deficient experiments with completely vitamin B_{12}-free diets. Hence, the authors considered that vitamin B_{12} in rats may be produced in vivo.

(3) In poultry droppings, vitamin B_{12}-producing Actinomycetes were always positively confirmed, but in any part of digestive canals of poultry, no Actinomycetes was ever dectable. Vitamin B_{12} in poultry droppings seems to be produced after excretion.

(4) Vitamin B_{12} in silk worms may be considered to be produced in vivo. Vitamin B_{12} was always detectable in egg, larva, pupa and moth, but completely negative in mulberry-leaves.

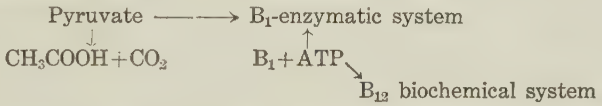
26. Biochemical studies on vitamin B_{12} (continued). Yoshikazu SAHASHI, Kiichi IWAMOTO, Masayuki MIKATA, Heiichi SAKAI, Taihei MIKI, Tadami AKATSUKA and Keiji HARASHIMA (Vitamins, **5**, 395, 415, 525, 609, 614, 615, 1952; **6**, 168, 291, 487, 1953)

27. Effect of vitamin B_{12} on energy metabolism. I. Yoshikazu SAHASHI, Kiichi IWAMOTO and Junzo HAYASHI (Proc. Japan Acad., **29**, 33-35, 1953; Journ. Biochem. Japan **40**, 245-249, 1953)

Recently, the present authors attempted to study the effects of vitamin B_{12} on energy metabolism. The results may be briefly summarized as follows:

(1) The total amount of vitamin B_1 and B_{12} decreased in blood on energy metabolism. The red blood cell counts in animals put on energy work also indicated a decrease and the diameter of red corpuscles increased, as seen in macrocytic anemia —the typical symptom of vitamin B_{12}-deficiency.

(2) The decrease of vitamin B_1 and B_{12} on energy work was completely protected by prophylactic injection of the vitamins at the beginning of experiment. Therefore, vitamin B_{12} seems to be essential to energy metabolism.



Variation of vitamin B_1 and B_{12} in blood effected by energy metabolism after injection of vitamin B_{12} was shown as follows.

	Total amount of B_1 after energy metabolism	Total amount of B_{12} after energy metabolism
Untreated control animal	−28.6%	−18.4%
Animal treated by B_1 only	−18.5	− 3.9
Animal treated by B_1 and B_{12}	− 8.7	− 2.1

28. Effect of vitamin B_{12} on the determination of vitamin D with rats fed on synthetic diets. Yoshikazu SAHASHI, Akihiko NAKAYAMA, Rentaro MATSUMOTO and Tadami AKATSUKA (Vitamins **6**, 175, 1953; Proc. Japan Acad., **29**, 33, 1953)

No anemia was recognized in rats in any case of the experiments with completely vitamin B_{12}-free diets. From this finding, no analytical accuracy of vitamin D with rats seems to be effected by vitamin B_{12}.

29. Studies on interaction of vitamin B_1 and sulfhydryl group in protein. Yoshikazu SAHASHI, Kyohei YAMASHITA and Tadami AKATSUKA (Vitamins **6**, 479, 1953)

In 1952, it was found by T. MATSUKAWA that vitamin B_1 and cysteine gave the mixed disulfide. Recently, the authors have attempted studies on "protein-S-S-B_1" type compounds, and it was observed by paper chromatography that papain-S-S-B_1 was prepared from vitamin B_1-disulfide and sulfhydryl group of active papain. Reduced glutathione (glutathione-SH) also reacted with vitamine B_1-disulfide to give glutathione-S-S-B_1.

The probable role of this reaction in intermediate metabolism was discussed.

30. Micro-chemical determination of vitamin A. Yoshikazu SAHASHI, Fumio

SAITO, Masanobu YOSHIDA, Akihiko NAKAYAMA, Kano MIYAZAKI and Keiji HARASHIMA (Vitamins **6**, 490, 589-593, 1953)

This investigation was carried out for the purpose of determining the daily requirement of vitamin A for Japanese. By BESSAY method, vitamin A in blood was estimated by ultra-violet irradiation. At first, home-made xylene and kerosene was previously refined and on the other hand, ultra-violet irradiation was studied by various domestic apparatus. The vitamin A content in fish liver oil and synthetic vitamin A solution was previously estimated by ordinary macro-method and then the home-made apparatus was tested. A satisfactory result was always observed in the determination of vitamin A in blood serum and blood plasma for research of Japanese nutrition.

31. **Studies on stability of multivitamin.** Yoshikazu SAHASHI, Akihiko NAKAYAMA, Toshitaka KAKUDA and Hideyo SUZUKI (Vitamins **5**, 622, 1952)

32. **Spectrophotometric determination of camphor-aldehydes.** Yoshikazu SAHASHI, Jinkichi UENO and Minoru MORI (Bull. Chem. Soc. Japan, **26**, 68-9, 1953)

Pure 2,4-dinitrophenylhydrazones of β (10)apocamphor-1-aldehyde and π (7 or 8)apocamphor-7-aldehyde were prepared in order to undertake spectrophotometric estimation of camphor-aldehydes with their standard crystalls. The absorption maxima were exactly determined in isoPrOH by a BECKMAN spectrophotometer to give $E^{1\%}_{1cm}$ $356_{m\mu}=658$ and $E^{1\%}_{1cm}$ $354_{m\mu}=678$ respectively.

The E value of the hydrazone in the solution to be tested was measured electrophotometrically, and the real amount of the camphor-aldehydes contained was calculated from the above standard E value.

33. **Studies on the nutritive value of grass proteins. Part 2. On the non-nitrogenous compounds in the leaf of radish.** Makoto KANDATSU and Tadahiko YASUI (Journ. Agr. Chem. Soc. Japan **27**, 66, 1953)

The authors designed to study the distribution of the nitrogen compounds in the leaf of radish and results obtained are as follows:

(1) About 65% of total nitrogen in the leaf is non-proteinous, especially nitrate and amino nitrogen.

(2) BARNSTEIN's method precipitates many non-protein nitrogenous compounds with true protein and most of which are amino compounds.

(3) From the precipitate by BARNSTEIN's method arginine, histidine, glutamic acid, aspartic acid, ammonia and purine-picrate (m.p. 235°C probably hypoxanthine-picrate) are isolated and leucine, alanine, cystine, phenylalanine, valine, serine and asparagine detected by paper partition chromatography.

34. **Synthesis of the coumarin derivatives and their antibacterial activities. Part 2. Synthesis of 3-acetyl-4,5-dihydroxycoumarin.** By Yoshio ISHII (Journ. Agr. Chem. Soc. Japan **26**, 510-3, 1952)

Two coumarin derivatives, substituted a hydroxy radical for the carbon atom at the 5th position of 3-acetyl-4-hydroxycoumarin were synthesized, to examine the effect of the negative substituent in the benzene nucleus of coumarin on the anti-bacterial activity of the coumarin derivatives.

The schema of the synthesis was illustrated by a chart. 2,6-dihydroxybenzaldehyde was prepared as a needle, m.p. 116°, from resorcin, introducing an aldehyde between the two hydroxyl groups by GATTERMAN's reaction, acetylating these hydroxy groups, and then oxidating the aldehyde to carboxylic group.

In this oxydation, the yield of diacetoxy-benzoicacid was found to be best when three times as much of $KMNO_4$ were used as it was theoretically calculated. 3-acetyl-4,5-dihydroxycoumarin, m.p. 158°, was obtained in 30% of the theoretical amount, condensing 2,6-diacetoxybenzoic acid chloride with sodium acetoacetic acid ester in the solution of the dried ether.

In this case 3-acetyl-4-hydroxy-5-acetoxycoumarin was scarcely obtained.

The antibacterial activities of these derivatives of coumarin will be published in the following paper.

35. Synthesis of coumarin derivatives and their antibacterial activities. Part 3. Synthesis of 3-acetyl-4-hydroxy-7-acetaminocoumarin and 3-acetyl-4-hydroxy-7-acetamino-8-bromocoumarin. Yoshio ISHII (Journ. Agr. Chem. Soc. Japan **27**, 310-3, 1953)

The coumarin derivatives, substituted an acetaminoradical at the position of the 7th carbon atom of 3-acetyl-4-hydroxycoumarin, were synthesized to examine the effect of the negative substituent on the antibacterial activity of the coumarin derivatives.

(1) 3-acetyl-4-hydroxy-7-acetaminocoumarin (III) and 3-acetyl-4-hydroxy-7-acetamino-8-bromocoumarin (III′) were prepared by a route, which was shown schematically, p-amino-salicylic acid (V) being used as the starting material.

The acetylation of (V) to prepare 2-acetoxy-4-acetamino-benzoic acid (VII) was performed by adding Ac_2O to the glacial acetic acid solution of (V) in the presence of $ZnCl_2$.

In this case, diacetate (VII), m.p. 183°, was obtained in a 86% yield 5% higher than the case of two-steps acetylation of (V), which was published by CARVALHO.

Through the acid chlorides (IX and IX′) of (VII) and its bromo-derivative (VIII), the direct cyclization with Na-acetoacetic ester was proceeded in dry ether, heating and stirring the mixture for ten hours, and gave the yields of 38% and 30%, respectively.

(III) was recrystallized three times from ethyl alcohol, in yellow prisms, and melted at 215°.

After two recrystallisations from aquous acetic acid, pure (III)′ was obtained as yellow needles, m.p. 225°.

When the equivalent mole of sodium acidchloride (IX, IX′) were used in such a direct cyclization as it is mentioned above, it was determined that the acetyl radicals of (III) and (III′) were not hydrolyzed.

(2) The results of the ultraviolet absorption spectra were shown in figure 1 and 2. It is very interesting that the curves of 3-acetyl-4,5-dihydroxycoumarin and 3-acetyl-4-hydroxy-5-acetoxycoumarin were very similar to that of the resorcinol, when it was displaced bathochromically.

The information of the bacterial activity of coumarin derivatives will be presented at a later data.

36. Studies on the microbial growth. Part 2. The statistical treatment of the bacterial growth curve. Akira MATSUYAMA (Journ. Agr. Chem. Soc. Japan **26**, 513-519, 1952)

The growth of the bacteria (*Escherichia coli* var. *communis*) was measured using the photoelectric turbidimeter, and the fitness to the logistic curve introduced by VERHURST was examined. The results were as follows ; (1) The variable y in the equation (IV), $y=L/[1+e^{-r(t-a)}]$, is defined as $y\equiv Y+\varepsilon$, where Y is the bacterial turbidity (log) established so that the turbidity at the time of the inoculation may be zero, and ε is $L/(1+e^{ra})$. Y is observable but ε is unknown. (2) The equation of finite differences, $Y_1+[(A+By_0+\varepsilon)/B]\cdot(Y_1/Y_0)+(-1+By_1+\varepsilon)/B=0$ where $A=e^{-rh}$, $B=(1-e^{-rh})/L$, $h=1$(hr.), derived from the equation (IV) is used to examine the graphical goodness of fitting to the logistic curve (IV). The points on this diagram of finite differences (Y_1 to Y_1/Y_0) are on a straight line, and this fact means the graphical good fitness. (3) Parameters L, r and ε are calculated directly from the value of P_1, Q_1, P_2 and Q_2, but the estimate of ε is adopted from the value of the $L/(1+e^{ra})$. By means of the interpolation, a is estimated. According to the method of the least squares presented by W. E. DEMING, the minimum value of the sum of the weighted squares was calculated. In order to determine the weights in y-coordinate, the standard deviations of the errors caused by the incubation and measuring were estimated experimentally. As the result of chi square test in which $\chi_0^2=\min S/\sigma^2=49.95$, degree of freedom$=8$, and $Pr\{\chi^2\geqq\chi_0^2\}<0.001$, it cannot be allowed that the growth curve of the bacteria follows the logistic curve (IV).

37. Studies on the microbial growth. Part 3. The growth of the bacteria in stationary cultures. Akira MATSUYAMA (Journ. Agr. Chem. Soc. Japan **26**, 638-642, 1952)

(1) Errors that are due to the variations between the multiplicate cultures of bacteria (*E. coli*) under the same condition distribute in the GAUSSIAN type, and the relationship between their standard deviation and the incubation time have the trend as well as that of the errors in the mean growth of the multiplicate cultures. The % errors in the latter are two- or threefold of that of the former, and both % errors in the stationary cultures reach the maximum at the midst of the logarithmic phase, following the decrease until half a maximal level. (2) The bacterial growth curve in the stationary phase by means of the stationary culture is seriously influenced by the ratio S/B (S: the surface area of the medium contacting with air; B: the volume of the medium). (3) Using these results, the errors of the microbiological assay by the turbidimetry have been discussed. (4) The bacterial growth curves under the various conditions of the stationary culture, were examined by means of difference diagrams, in order to find whether or not they would well fit to the logistic curve $y=L/[1+e^{-r(t-a)}]$, and the best fitted growth to the logistic curve including the lag phase was obtained in the most suitable medium for the bacterial growth.

38. Studies on the microbial growth. Part 4. The methods for determining the viable count of the bacteria. Akira MATSUYAMA (Journ. Agr. Chem. Soc. Japan **27**, 7-11, 1953)

The viable counting of the bacteria (*E. coli*) was studied by the plating and "capillary tube methods" examining especially (1) the using technic of the pipette, (2) the distribution type of the pipetting error, (3) the error in the tube counting and (4) the comparison with the hemocytometer method. The results were as fol-

lows: (1) Between the colony counts obtained by the consecutive uses of the same pipette from the same bacterial suspension, there are no significant differences. But, when the dilution of the bacterial suspension is performed by the repeating uses of the same pipette, the colony counts increase in proportion to the times of repeating uses. (2) In the cases of plate and tube countings, it has been shown that the errors of the viable counting which is due to pipetting, distribute generally in POISSON type, and when the mean count is large (more than 30), it can be allowed to say that the error of the counting distributes approximately in GAUSSIAN type. (3) In the analysis of the error variance of the tube counting, the variances between dilution series show no significant differences. (4) Mean counts obtained by the hemocytometer and tube counting from the same bacterial suspension, are shown in Table 1.

Table 1. Mean counts obtained by hemocytometer and tube counting.

	Total counts by hemocytometer	Viability counts by tube counting
Logarithmic phase	$3.74\times10^{7}/ml$	$3.51\times10^{7}/ml$
Stationary phase	8.80×10^{8}	3.36×10^{8}

As to the mean counts and variances, there are no significant differences between the plating and capillary tube methods (Table 2).

Table 2. Mean colony counts and variance obtained by plate and tube counts.

	Number of sample	Mean counts	Variance
Plate counts	16	312.1/*ml*	345.3
Tube counts	16	318.1	425.1

The capillary tube methods' have been named by T. YANAGITA and H. TAMIYA (University of Tokyo) and here the viable counting by this method is called the tube counting.

39. The biochemistry of bakanae fungus. Part 25. The physiological action of gibberellin. III. Yusuke SUMIKI (Journ. Agr. Chem. Soc. Japan **26**, 393, 1952)

All young plants which were treated with a definite concentration of gibberellin grow abnormally tall and slender. In order to dissolve this phenomenon, the physiological actions of gibberellin were examined, firstly, comparing with those of plant growth hormones such as heteroauxin. The gibberellin used in all experiments of this paper is a crystalline gibberellin A,d.p. 242-4°. It was identified by the following experiments that there was a big difference in the mode of action between gibberellin and auxins.

(1) It did not induce the cell division of callus of *Bryophyllum* and, also, the callus formation of soy bean, sunflower and tomato.

(2) It did not suppress the formation of lateral buds in kidney bean and etiolated pea.

(3) It did not induce the epinacy of tomato.

(4) It was found to be entirely inactive in the avena standard and pea tests.

(5) It had been reported in the previous paper that it had hindered the growth of roots of rice seedlings, but it was found to have no such effect as promoting or hindering on the development and the branching of *Avena* roots in an extent of concentrations of 0.02-10 mg/l.

Secondly, the cells of stamen hairs of *Trdescantia roflexa* were cultured in th agar plate containing 2% of saccharose and various concentrations of gibberellin t observe the mitosis of cells and the elongation and enlargement growths of devide cells. The results were as follows:

(1) The gibberellin was found to be hardly recognizable as a promotion sub stance for mitosis, but it never hindered the occurrence of mitosis even in such high concentration of 0.01% as to make the rice seedlings dead. This fact show that gibberellin is a kind of plant hormone, but not a poisonous substance agains protoplasma.

(2) It was certainly and clearly observed that its effect on elongation growt appeared more remarkably on the embryonal cells than the adults and also, more a the basic part than the front part in each cell under the concentration of 0.0005 0.00001%, resulting in the formation of the cells shaped in bottle-like pattern. Fron this former fact and the fact that the growth of cell membrane in the tissue occurre even in the youngest cells immediately after their division which did not yet forn the chlorophyll, it is confirmed cytologically that the physiological action of gib berellin is quite indifferent to the assimilation of chlorophyll.

Concerning the reason of the latter fact, the following affairs are assumed One stamen hair is composed of cells standing in only one line and each cell contact each other only with septum. Therefore, the membrane of each cell is able to mak freely its elongation growth as well as its enlargement growth besides this contact ing portion. In spite of this freedom, the fact that the basic part of each cell makes especially, more enlargement growth than the front part resulting in the bottle like shape, is considered to be the cellulose micells are accumulated too much i the basic part. The similar phenomena to this fact are observed in the stem growt of monocotyledon, i.e., the stem growth depends on the reproduction of young tissu located just in upper part of each node. It is considered that there is such a polarity also, even each cell. But, in the case of the divided tissue in plant body, the enlarge ment growth is limited more than the elongation growth by the resistance betwee each tissue and, as the result, each cell is obliged to make only the elongation growt showing the characteristic abnormal phenomenon.

Thirdly, the percentages of elongation growth and of reproduction were examin ed quantitatively using soybean, sunflower, tomato and lemna as the testing plants.

The author wishes to express his cordial appreciation to Dr. G. ASHIDA, Depart ment of Botany, University of Kyoto and Dr. B. WADA, Department of Botany University of Tokyo, who carried out the investigations.

40. The biochemistry of bakanae fungus. Part 26. The chemical constitutio of gibberellin. Part 6. Yasuo SETA and Yusuke SUMIKI (Journ. Agr. Chem. Soc Japan **26**, 508, 1952)

In the previous paper of this series, we assumed that the position of the carbony group in gibberic acid should be in its side chain but not in its mother nucleus, adjust ing to the terminal methyl group, from the results of the oxidation of gibberic aci with SeO_2.

In this paper, the gibberic acid was treated with hydroxylamine and its oxim wa prepared. This oxim was treated with phosphorus pentachloride to perform BECK MANN's rearrangement and then the reaction mixture was hydrolysed with concen

trated hydrochloric acid. As the hydrolytic products, the methylamine (0.7 moles), a dibasic acid, $C_{16}H_{18}(COOH)_2$, (0.3 moles) and a substance, d.p. 305-7° (0.2 moles) were obtained. The methylamine was identified as its picrolonate and a dibasic acid was identified from the results of the elementary analysis and the molecular weight determination. $C_{18-19}H_{21-23}O_3N$ was assumed as the molecular formula of the crystalline substance decomposing at 305-7°.

From the facts above described we confirmed that the position of the ketone group in gibberic acid must be located not in the fluorene nucleus but in one of the side chains, adjusting to the methyl group of its side chain and, moreover, the terminal radical of this side chain must be a methyl group but not a carboxyl group.

41. Studies on the inhibition of the autoxidation of fats and oils. Part 2. On the antioxydant property of caffeic and dihydrocaffeic esters. Saburo TAMURA, Kazuhiko OHKUMA and Tadahiko HAYASHI (Journ. Agr. Chem. Soc. Japan **26** 410-2, 1952)

To examine the relation between the structure of o-dihydroxyphenylalkanoic acid and their antioxydant property, caffeic and dihydrocaffeic esters were synthesized and their effectiveness in stabilizing vitamin A in cod-liver oil was determined by means of BECKMANN spectrophotometer. As the standard antioxydant ethyl gallate was added to liver oil in concentration of 0.05% and other compounds to be tested were incorporated with it on an equivalent molecular basis. The liver oil was shaken on rotatory shaker (190∼200 *r*/min) at 24°. The stability value of the control (i.e., the time in days for 50% loss of vitamin A in the absence of antioxydants) was 11 days. The stability value of ethyl protocatechuate was 13 days, but introduction of c=c double bond between phenyl and carbonyl groups as in caffeic esters led to marked enhancement in effectiveness to 32 days, moreover, hydrogenation of the double bond as in dihydrocaffeic esters increased activity to 35 days but activity of these compounds was inferior to that of ethyl gallate (stability value, 46 days).

42. Studies on the inhibition of the autoxidation of fats and oils. Part. 3. On the antioxydant property of hydroxycoumarines. Saburo TAMURA, Kazuhiko OHKUMA and Tadahiko HAYASHI (Journ. Agr. Chem. Soc. Japan **26**, 413-4, 1952)

In order to study the relation between the chemical structure and the antioxydant property, 12 hydroxycoumarin derivatives were synthesized and their activities in stabilizing vitamin A in cod-liver oil were examined, using the same method described in the previous paper. The results were as follows:

(1) 3,4-Dimethyl-esculetin (mp. 260°), 3-iso-propyl-4-methyl-esculetin (mp. 280°), 3,4-dimethyl-daphenetin (mp. 278°) and 4-methyl-3,4-dihydro-esculetin (mp. 155°) were new compounds.

(2) 6-Hydroxy-4-methyl-, 7-hydroxy-, 7-hydroxy-4-methyl-, 5,7-dihydroxy-4-methyl-coumarins were found to be inactive.

(3) The derivatives of esculetin were found to be more active than those of daphenetin in general.

(4) The alkyl-substitution at the 4-position of esculetin caused no enhancement in activity but the substitution at the 3-position increased activity.

(5) Among all hydroxycoumarins which were tested, 3,4-dimethylesculetin was the most effective but its activity was inferior to that of methyl caffeate.

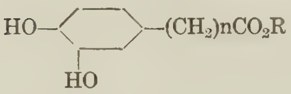
(6) 4-Methyl-3,4-dihydro-esculetin prepared by the hydrogenation of 4-methyl-

esculetin showed marked effectiveness and its activity was almost the same as that of ethyl gallate.

43. Studies on the inhibition of the autoxidation of fats and oils. Part 4. Synthese of some ω-(3,4-dihydroxyphenyl) alkanoic esters. Saburo TAMURA, Kazuhiko OHKUMA and Tadahiko HAYASHI (Journ. Agr. Chem. Soc. Japan **27**, 318-22, 1953)

It was reported in the previous paper that several dihydrocaffeic esters were synthesized and were found to have the marked antioxidant activity. The present work was undertaken to synthesize ω-(3,4-dihydroxyphenyl) alkanoic esters, the related compounds to caffeic esters, in seeking further light upon the relation between the chemical structure and the antioxidant activity. These new esters are listed in the following table 1.

Table 1.



Substuents		mp.
n	R	
1	Me	34~36°
2	Et	43~45°
3	Me	38~40°
4	Et	35~37°
5	Me	66~67°
5	Et	49~50°
5	n-Pr	45.5~47.5°

To prepare homoprotocatechuic ester veratric aldehyde was treated with hippuric acid, and the condensate, α-benzoyl-amino-β-veratryl-acrylic acid azlacton was hydrolysed to obtain homoveratryl-pyruvic acid which was followed by dimethylation with HBr.

The resulting acid was esterified by the usual way. γ-(3,4-Dihydroxyphenyl)-butyric acid was prepared in the following way: Veratrol was condensed with succinic anhydride in the presence of $AlCl_3$, the resulting β-veratroylpropionic acid was treated with Hl for for demethylation, reduced to γ-(3,4-dihydroxyphenyl)-butyric acid by the CLEMENSEN reduction, and then esterified. δ-(3,4-dihydroxyphenyl)-valeric ester was synthesized by the following 4 steps. γ-Veratroyl-butric acid was obtained by the condensation of veratol with glutaric anhydride. Then the reduction with the CLEMENSEN method, the demethylation by Hl and esterification were proceeded successively. ε-(3,4-dihydroxyphenyl)-caproic ester was prepared through the CLEMENSEN reduction, the demethylation by Hl and the esterification of the condensation product of veratrol with polyadipic anhydride in the presence of $AlCl_3$. Their antioxidant activity will be published in the following paper.

44. Studies on the inhibition of the autoxidation of fats and oils. Part 5. Antioxidant property of some ω-(3,4-dihydroxyphenzyl) alkanoic esters. Saburo TAMURA, Kazuhiko OKUMA and Tadahiko HAYASHI (Journ. Agr. Chem. Soc. Japan **27**, 323-7, 1953)

To make an approach to the elucidation of the relation between a chemical structure and an antioxydant activity, the activities of some ω-(3,4-dihydroxyphenyl) alkanoic esters were examined by means of the Active Oxygen Method.

The alkanoic ester were synthesized by the authors and the fresh lard was used as the substrate, the rancidity of which was easily detectable for its rancid odour.

The results of the experiment were as follows:

(1) At protocatechuate was found to be almost ineffective but an introduction of methylene groups between phenyl and carboalkoxyl groups of protocatechuic ester caused the marked enhancement in activity. The activities of most of these compounds were proved superior to that of ethyl gallate.

(2) Among these compounds, methyl ε-(3,4-dihydroxyphenyl)-caproate which had 5 methylene groups was the most effective and γ-(3,4-dihydroxyphenyl)-butyrate having 3 methylene groups the next.

(3) Concerning the alkyl groups of esters, it was found that the oder of their activities was Me < Et < n-Pr, regarding solely to ε-(3,4-dihydroxyphenyl)-caproic ester.

(4) It is noteworthy that the protocatechuic ester which was reported by OSHIMA to be very effective was found to be almost ineffective to the cod liver oil and lard used by the authors as the substrates.

45. Studies on the high potency producing molds of penicillin. IV. Studies on the newly isolated strains. Shigeo ABE, Tatsuji SHIGA and Yasushi NAKANO (Journ. Antibiotics **8**, 433-439, 1952)

About 1,000 strains of *Penicillia* were isolated from about 3,000 samples obtained from soils of different places and from other materials. The penicillin production of these strains were tested.

One of these, designated as 3 A (1185) was found to produce penicillin of 263 u./cc in corn steep liquor medium added with 0.4% phenylacetic acid.

One hundred and eighty-two strains of high potency producer can be divided into 13 types macroscopically, which are classified into following species: Asymmetrica—*P. citrinum* THOM, *P. notatum* WESTLING, *P. cyaneo-fulvum* BIOURGE, *P. chryosogenum* THOM, and *P. viridicatum* WESTLING.

Strain No. 81 (411) produces 50-60 u./cc penicillin marked by rapid-growing colonies that are rather dark Blackish green, Dusky yellowish green (XLI) in 4 to 20 days at 25° and 30°C on CZAPEK's and steep agar, produces abundant colorless exudate, and colony reverse uncolored or in pale yellow shades. Therefore, this strain was recognized to be a new strain belonging to the species P. notatum WESTLING.

By microscopic observation of few variants of 3 A induced by ultraviolet irradiation prolonged or slender sterigmata and metulae, one anastomosis of conidiophores, or divarocate penicillus, larger and smaller conidia were recognized.

46. Studies on the heat resistance of bacterial spores. Part 3. Effects of sugars in the subultcure media on the survival times of *Bacillus natto*. Mikio AMAHA (Journ. Agr. Chem. Soc. Japan **26**, 306, 1952)

The spores of *Bac. natto* were suspended in neutral phosphate buffer and heated at 100°C, and then subcultured by various media. The yeast-extract broth, liver-extract broth, and glucose broth showed survival time twice as long as plain nutrient broth.

The effects of adding various carbohydrates and organic acids were examined. Fructose, mannose, galactose, sucrose, maltose, and soluble starch were found to have the increasing effect on the survival time of spores of *Bac. natto* to the same extent as glucose, but other eight sugars have had no effects. The addition of

pyravate or α-glycerophosphate to nutrient broth also increased the survival time, but the addition of organic acids such as lactic, acetic, and succinic had no effects.

It was found that in a synthetic medium, which is composed of 18 amino acids, 9 vitamines, and salts, the vegetative cells of *Bac. natto* grew well without the addition of glucose, whereas the spore could not grow without glucose (carbohydrates). And it was also found that the kind of carbohydrates which were effective in increasing the survival time of the spores of *Bac. natto* was the same with that which served as the energy source and which enabled the spores to grow in the synthetic medium. The minimum effective concentration of glucose in increasing the survival time of the spores was between 10^{-5} M and 10^{-6} M, and the same order of glucose concentration also has limited the growth of spores of the strain in the synthetic medium.

It should be noted that when subcultured by glucose broth, the survival time at each spore concentration was commonly about twice that of plain nutrient broth, throughout a wide range of spore concentration.

The mechanism of spore survival time increasing activities of glucose were discussed.

47. Studies on the heat resistance of bacterial spores. Part 4. Kinetics of the death reaction of spores by heat. Mikio AMAHA (Journ. Agr. Chem. Soc. Japan **26**, 339, 1952)

The phenomena of death by heat of spores of three aerobic bacilli, *Bacillus natto*, *Bacillus megatherium* and *Bacillus mycoides*, were pursued and discussed. The spores were suspended in neutral phosphate buffer and heated in a glass tube and then subcultured or plated out by nutrient broth (agar) and glucose broth (agar).

The survivor curves obtained by plotting the logarithm of the number of survivors against the heating time were nearly straight, however the death rate constants calculated by the equation for monomolecular chemical reaction decreased with the time of exposure. It should be noticed that the survivor curves of various initial spore concentrations, though they are linear, do not parallel each other and the slope of the curves becomes larger as the initial spore concentration decreases. This fact will mean that the death rate constant K is not a fixed value for spores of a certain strain. Using the general relation between spore concentration and survival time obtained in the previous paper it was shown that the death rate constant (K) and the initial spore concentration (N) can be related by the following equation,

$$K = C \times \left(\frac{1}{N}\right)^n \times \log N$$

where C and n are constants.

The temperature coefficients of the death of spores of three aerobes caused by heat in neutral phosphate buffer were computed as follows (between 95° and 100°C). *Bac. natto*....$Q_{10}=16.97$, *Bac. megatherium*....$Q_{10}=6.25$, *Bac. mycoides*....$Q_{10}=13.17$.

The activation energy and the thermodynamic functions, such as E_{Ar}, ΔH, ΔS, ΔF, about the death of spores of each strain were evaluated per one spore.

The values were in the same order with those of protein denaturation.

From these results, it was concluded that the spore of bacteria would lose its ability to reproduce, i.e., die, when a single protein molecule in the cell was denatured by heat as previousy stated by O. RAHN.

48. Studies on the heat resistance of bacterial spores. Part 5. Influences of vitamins and amino acids in the synthetic subculture media on the survival time.

Mikio AMAHA (Journ. Agr. Chem. Soc. Japan **26**, 420, 1952)

Effects of vitamins in the complete synthetic medium on the survival time of the spores were tested by omitting each vitamin singly or by adding only one or two vitamins in replacement of the vitamin mixture. The result was that the presence of thiamine and pyridoxal in addition to biotin (biotin is essential for the growth of the spore of *Bac. natto*) was effective in elongating the survival time of this strain.

Effects of the kind of amino acid as a sole nitrogen source on the survival time were studied by adding each amino acid as NH_2-nitrogen 0.02% in replacement of the amino acid mixture of original medium. The varied length of survival time is obtained according to the amino acid, and it would be noted that the kind of amino acids which are known to be converted easily or directly to glutamic acid gave the longer time and the kind of amino acids which cannot be converted easily to glutamic acid gave the shorter survival time.

From the results obtained, it was concluded that the cause of the death of bacterial spores by heat is the destruction of some of the protein molecules which control each metabolic systems which are necessary to synthesize cell materials from the nutrients given. It was shown, however, that the protein molecules are not "gene".

49. Nutritional requirements of vegetative cells and spores of aerobic spore-forming bacilli. Mikio AMAHA and Kin-ichiro SAKAGUCHI (Journ. Agr. Chem. Soc. Japan **26**, 353, 1952)

The nutritional requirements of vegetative cells and sports of six aerobes, *Bacillus natto*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megatherium*-I and -II, *Bacillus mycoides* and *Bacillus cereus* were studied by using a complete synthetic medium. Four of the six strains could not grow in the complete medium containing 2.0% glucose. However, when the concentration of the baseal medium was diluted to one-fourth of the original or when the concentration of glucose was reduced to 0.5% both the vegetative cells and spores of all six strains showed good growth. Thereafter, we have used, as the basal medium, the one-fourth concentration of the original medium with 0.5% glucose. The spores of all strains except that of *Bac. cereus* did not grow without glucose in the medium. The ability to utilize ammonia has been tested by the medium in which the amino acids were replaced by ammonium acetate.

Purine and pyrimidine bases were not necessary for the growth of vegetative cells and spores of all strains. Vitamin requirement tests have showed that only biotin is necessary for growth of *Bac. natto* and *Bac. mycoides*, and no vitamins are needed by the other four strains. It should be noted that there was no difference between vegetative cells and spores of each strain.

Omission of each single amino acid from the mixture of eighteen amino acids in the basal medium showed that vegetative cells of all strains and spores of the two strains (*B. subtilis* and *B. mycoides*) had no "indispensable" amino acid. However, the omission of DL-isoleucine for the spores of *B. natto* the omission of DL-valine for the spores of *Bac. cereus*, the omission of DL-valine for the spores of *Bac. cereus*, the omission of DL-alanin or DL-threonine for the spores of *B. megatherium* (II) completely inhibited the growth of each.

Utilization of a single amino acid was studied using a medium which contains a certain amino acid as the sole N-source instead of the amino acid mixture. Among the nineteen amino acids tested, the five, L-glutamic acid, L-arginine, L-asparagine,

L-hitidine, and L-proline, supported fair growth of the vegetative cells and spores of all strains. No growth or only scanty growth of all strain was obtained by DL-threonine, L-cystine, DL-methionine, DL-tryptophan, or L-tyrosine. When glycine, DL-alanine, DL-valine, L-leucine, DL-isoleucine, or DL-serine, was added as the sole N-source, it was frequently found that only the vegetative cells showed growth and the spores of the same strain failed to grow. The differences in the amino acid requirements and in the ability to utilize a single amino acid between the vegetative cells and the spores of each strains seemed to be attributed not only to differences in the synthetic ability but also to differences in the susceptibility to the toxicity of certain amino acids.

50. Studies on variations of penicillin producing molds. Part 11. Biochemical genetical studies on the yellow pigments losing mutation of *Pen. chrysogenum* Q 176 to pigmentless sultant Q 176 ARIMA et OGASAWARA. Kei ARIMA, Kazuo KOMAGATA and Hideo NAKAMURA (Journ. Agr. Chem. Soc. Japan **27**, 339, 1953)

For the purpose of elucidating the mechanism of biochemical genesis of the yellow pigments losing mutation of *Pen. chrysogenum* Q 176 to pigmentless sultant *Pen. chrysogenum* Q 176 ARIMA et OGASAWARA, cultural conditions of the yellow pigment production of *Pen. chrysogenum* Q 176 by extinction of culture media by Pulfrich photometer, and paper-chromatography of the pigments were examined. Effects of carbohydrate sources, nitrogen sources, C:N ratio, rate of aeration, initial pH of culture media, each representative media for penicillin production, cultural temperature, inorganic salts on the production of pigments, and inhibitive action of NaCl, relation between pH and extinction of the pigments were examined.

From the above results, the pigment production and penicillin production seemed to be parallel, but unlike the case of penicillin, pigments did not run down rapidly after reaching its peak, but keeps its level.

By selection of cultural conditions, it was possible to increase pigment production 2 or 3 folds of the control, but not so much as 10 folds or more as in the case of penicillin production.

Next, paper chromatographic study of the yellow pigments, especially the experimental conditions were examined. Final conditions were as follows: Toyo filter paper No. 50, size 2 × 40 cm, pretreated with SÖRENSEN phosphate buffer solution at pH 7.7, developing solvent (butanol 90 cc, isopropanol 10 cc, water 18 cc, (80% saturated), developing hour 17-18 hrs., room temperature.

The fractionated spots on the paper stripe could be detected by the naked eye, $FeCl_3$ reaction or ultraviolet absorption spectrum of the extract from the spot by BECKMAN's quartz spectrophotometer.

51. Studies on variation of penicillin proudcing mold. Part 12. Biochemical genetical studies on the yellow pigments losing mutation of *Pen. chrysogenum* Q 176 to pigmentless sultant *Pen. chrysogenum* Q 176 ARIMA et OGASAWARA (2). Kei ARIMA, Hideo NAKAMURA and Kazuo KOMAGATA (Journ. Agr. Chem. Soc. Japan **27**, 345, 1973)

By paper chromatographic studies, using the conditions decided in the previous report, on the yellow pigments produced by *Penicillium chrysogenum* Q 176, it was ascertained that only two pigments were proudced by this mold and that these two

spots had the R_f values of 0.65-0.66 and 0.49-0.50 respectively.

These two pigments were identified as sorbicillin and penitrinic acid by comparision with the R_f values and ultraviolet absorption spectrum of the extract of each spot to those of crystalline sorbicillin and penitrinic acid, which is kindly given by Dr. D. G. CRAM of California University, Dr. F. H. STODLA of NRRL and Dr. T. ODA of Yashima Company and for whom the authors wish to express their sincere thanks.

52. The microbiological determination of amino acids in human milk, cow's milk and amino acid injections. Jiro KIRIMURA and Gakuzo TAMURA (Journ. Agr. Chem. Soc. Japan **26**, 603, 1952)

The amino acid composition of human and cow's milk and several amino acid intravenous remedies now manufactured in Japan have been determined by microbiological method.

Difference of composition of some essential amino acids in human and cow's milks are as follows; the concentration of protein in both milks was corrected to 1.5, 1.45% respectively and compared: The former is poor in methionine (57% of the latter) and also in phenylalanine, valine. As to non-essential amino acids, the former contains twice in tyrosine and less amounts of serine, glycine, glutamic acid. But as a whole, it can be concluded that cow's milk has not less nutritional value than human milk in amino acid composition.

The authors compared their results of human and cow's milk with other data hitherto published, and found the notable differences in tyrosine and glycine content.

53. The decomposition of d-tartarate by the micro-organism. Part 1. Masayasu NOMURA (Journ. Agr. Chem. Soc. Japan **27**, 46, 1953)

Though d-tartaric acid is widely distributed in nature, its metabolic behavior has been hitherto scarcely known. To clarify this problem the decomposition of d-tartarate by one strain of *Pseudomonas* isolated from the soil was studied.

Succinic and acetic acids were isolated and identified as the decomposition products of d-tartaric acid by resting cells. Furthermore, it was confirmed from the quantitative studies that anaerobic decomposition of d-tartaric acid was performed according to the following equation:

$$3\begin{array}{l}CH(OH)COOH\\ |\\ CH(OH)COOH\end{array} \rightarrow 2\,CH_3COOH + \begin{array}{l}CH_2COOH\\ |\\ CH_2COOH\end{array} + 4\,CO_2 + 2\,H_2O \quad \ldots\ldots\ldots \; (1)$$

and it was suggested that this reaction may proceed via oxalacetate as follows:

$$3\begin{array}{l}CH(OH)COOH\\ |\\ CH(OH)COOH\end{array} \longrightarrow 3\begin{array}{l}CH_2COOH\\ |\\ CO\text{-}COOH\end{array} + 3\,H_2O$$

$$2\begin{array}{l}CH_2COOH\\ |\\ CO\text{-}COOH\end{array} \longrightarrow 2\,CH_3COCOOH + 2\,CO_2$$

$$2\,CH_3COCOOH + 2\,H_2O \rightarrow 2\,CH_3COOH + 2\,CO_2 + 4\,H$$

$$\begin{array}{l}CH_2COOH\\ |\\ CO\text{-}COOH\end{array} + 4\,H \longrightarrow \begin{array}{l}CH_2COOH\\ |\\ CH_2COOH\end{array} + H_2O$$

Standard free energy of formation of bivalent d-tartaric ion at 25°C was calculated to be −242,990 cal./mol. and the free energy change in the above reaction was calculated to be −64,400 cal. or −21,500 cal./mol. of tartarate.

54. Studies on the decomposition of citrate by micro-organisms. Part 1. On the organic acids metabolism in *Bact. succinicum*. Hajime TAKAHASHI and Masayasu NOMURA (Journ. Agr. Chem. Soc. Japan, **26**, 99, 1952)

The oxidation of various organic acids belonging to the tricarboxylic acid cycle by the intact cells of *Bact. succinicum* was studied. In anaerobic condition this microbe decomposed citrate into succinate, acetate and carbon dioxide.

The oxidation of acetate by pantothenate (PaA)-deficient cells was markedly increased by PaA-assimilation, while the effect of PaA assimilation on the anaerobic citrate decomposition was not recognized.

Malonate inhibited greatly the oxidation of acetate and pyruvate but not of succinate by washed cell suspension. This inhibition was overcome by the addition of small amounts of fumarate. Furthermore, the succinate oxidation of the cell-free preparation obtained from the cells was completely inhibited by malonate. Therefore, the observed inhibition for acetate and pyruvate oxidation is due to inhibition for succinodehydrogenase.

When acetate was oxidized in the presence of malonate the accumulation of succinate was not recognized. Thus the THUNBERG-WIELAND's scheme for the acetate oxidation was excluded in this bacteria.

The presence of tricarboxylic acid cycle was confirmed not only from the above facts but also from the inhibition experiments with streptomycin, 2,4-dinitrophenol, monofluoroacetate and arsenite.

α,α'-Dipyridyl was found to inhibit anaerobic decomposition of citrate, but the oxidation of citrate was stable to this reagent. Thus the anaerobic decomposition of citrate involved the different process from that in aerobic state.

Dried cell preparation was found to contain the enzyme which catalyzed the anaerobic decomposition of citrate.

55. Studies on the natural variation of Koji-molds (*Asp. oryzae* and *Asp. sojae*). Part 2. Cytological studies on the natural variation of Koji-molds. Kin-ichiro SAKAGUCHI and Chiyoko ISHITANI (Journ. Agr. Chem. Soc. Japan, **26**, 85, 1952)

The constant strain of the Koji molds, S- or F-types, form conidia with comparatively uniform sizes; i.e. $S=3\text{–}5\,\mu$ and $F=4\text{–}6\,\mu$. The inconstant strains, on the contrary, produce conidia rich in variety of sizes, including those with extraordinarily large diameters ($3\text{-}10\,\mu$ or more).

The conidia of the constant strains have been found to contain 1-4 nuclei, the majority containing two nuclei. The conidia of the inconstant strains, however, are exceedingly multinucleate (8-20 or more) and large in diameters.

In the constant strains, one nucleus moves into conidium from apex of the sterigmata at the beginning of the conidium formation, and divides at once into 2-4 probably through mitotic divisions.

While in the inconstant or variable strains, more than 2 conidia migrate from sterigmata into newly formed conidium. Those conidia become multinucleate (8-20 or more) promptly through repeated divisions of nuclei.

As to the migration of nuclei from vesicle, which is conspicuously multinucleate, into sterigmata, the authors have recognized that in the constant strains always only one nucleus migrates into sterigmata while in the inconstant strains at least 2-4 simultaneously, the sterigmata becoming much more long and broad consequently.

From the facts above shown it might be assumed that the variability of the inconstant strains of the Koji molds chiefly depends upon the multinucleate property of the conidia.

56. Studies on the natural variation of Koji-molds (*Asp. oryzae* and *Asp. sojae*). Part 3. Studies on the mechanism of natural variation based on heterocaryosis. Kin-ichiro SAKAGUCHI and Chiyoko ISHITANI (Journ. Agr. Chem. Soc. Japan **26**, 279, 1952)

Anatomosis occurs between variants and between different strains of Koji-molds.

Anastomosis takes place often between conidia, between germ-tubes and between mature hyphae, accompanied by vigorous nuclear division and nuclear migration through connection tube or fusion bridge.

When the constant types S and F are grown together in mix-culture, they produce intermediate type X. In pedigree-culture by successive single spore method of the X-type, they again separate into all 3 types, namely, X, S, and F.

The constant type or homo-type S and F or conidia are comparatively uniform in sizes and have 1-4 nuclei.

The conidia of inconstant type or hetero-type X, which occur by mix-culture, are rich in variety of sizes and multinucleate.

By mix-culture between different strains of Koji-molds, they also give rise to an interstrainic dicaryophytic hybrids.

From the above facts and the findings hitherto reported[1)] it may be concluded that the natural variations of Koji-Molds (*Asp. oryzae* and *Asp. sojae*) are to greater extent responsible to the heterocaryosis. And the resultant heterocaryosis produce generally conidia of much greater diameters than those of the parent strains, a phenomenon similar to heterosis, through which the heterocaryosis are very easily detectable.

57. Studies on the natural variation of Koji-molds (*Asp. oryzae* and *Asp. sojae*). Part 4. Studies on heterocaryotic strains induced by irradiation of ultraviolet ray. Kin-ichiro SAKAGUCHI and Chiyoko ISHITANI (Journ. Agr. Chem. Soc. Japan **26**, 435, 1952)

The S- and F-type strains of *Asp. oryzae*, which are the constant natural variants of the aX type, have been irradiated with ultraviolet rays and the resultant mutants have been investigated cytologically with the following results.

The following mutants have been isolated from the S-type. 1. Normal; 2. Restricted; 3. Sterile; 4. Nitrate non assimilative; 5. Nitratophobe; 6. Heavy; 7. Olive; 8. Brown; 9. Albino; 10. Floccose; 11. Yeast; 12. Maroon-exudate from the F-type; 1. Normal; 2. Restricted; 3. Sterile; 4. Nitrate non assimilative; 5. Nitratophobe; 6. Normal (S); 7. Restricted (S); 8. Brown; 9. Yellow; 10. X(FS).

The conidia of the mutant types, Normal, Restricted, Sterile, Heavy, Yeast, and Maroon-exudate, have been found to contain 1-4 nuclei like their parent strains. The morphological characteristics of those mutants have remained stable even through eight generations of successive cultures, with a few exceptional cases in the types of Sterile, Yeast, and some of the Restricted which gave rise to reversions to the parent types.

(1) SAKAGUCHI and C. ISHITANI: J. Agr. Chem. Soc. Japan **26**, 85, 1952.

The conidia of the other mutant types, Nitratophobe, (or non the nitrates or difficulty assimilative) Olive, Floccose, Brown, and FS, are multinucleate (1-8 or more) and generally larger in the diameters (so-called "Magansporus type"). These types are very liable to give rise at the first generation of single spore isolated to the parent types and to the X-type which is the original strain for the parents, S- and F-types.

From the above facts it is obvious that from the constant strains with homocaryotic conidia heterocaryotic strains can be produced through the UV-irradiation and that the resultant strains segregate into parent type or even to the original type.

One of the mutants, the Albino type, (S 16 A) frequently produces conidia which have not any nucleus. Such conidia do not generally germinate or the germ tube does not elongate when the germination occurs.

58. On the microbiological assay of vitamin B_{12}. Comparative study of various assay organisms. Heiichi SAKAI, Shigeru MIYAZAWA and Osamu TERADA. (Journ. Agr. Chem. Soc. Japan **27**, 377, 1953)

Four test organisms for B_{12} assay, namely, *L. lactis Dorner, L. leichmannii, Euglena gracilis* var. *bacillaris, E. coli* mutant were compared from the standpoint of bioassay of B_{12}. As for the stability of B_{12} responsing quality, *E. coli* mutant seems to be inferior to other three organisms. The authors calculated the error range of B_{12} standard curve of these organisms and concluded that the accuracies of them are almost the same (error range is about 10-15%). As a result of factorial analysis by statistical method, the authors found that autocalving condition is the most influential factor for the growth of lactobacilli, and for *Euglena*, the size of inoculation is the most influential one. The inhibition effect of antibiotics present in samples was studied. Two strains of lactobacilli are sensitive to the inhibitory effect of penicillin and bacitracin, but *Euglena* can grow normally in the presence of the antibiotics.

59. Studies on the decomposition of citrate by micro-organisms. Part 2. On the acids metabolism by the dried cell preparation of *Bact. succinicum*. Hajime TAKAHASHI (Journ. Agr. Chem. Soc. Japan **27**, 84, 1953)

In the previous paper, the organic acids metabolism by the intact cells of *Bact. succinicum* had been reported. In the present paper, the metabolic activities of the dried cell preparation was studied.

Two types of dried cell preparation were prepared, one having the activity of acetate oxidation and the other being deprived of the activity of acetate oxidation almost completely.

By using the latter preparation, the author found that all the acids belonging to the tricarboxylic acid cycle except α-ketoglutarate were rapidly oxidized to acetate. Since the oxidation of each mole of these substrates stopped at the stage of one mole of acetate, it was very favorable that the oxidation took place via tricarboxylic acid cycle.

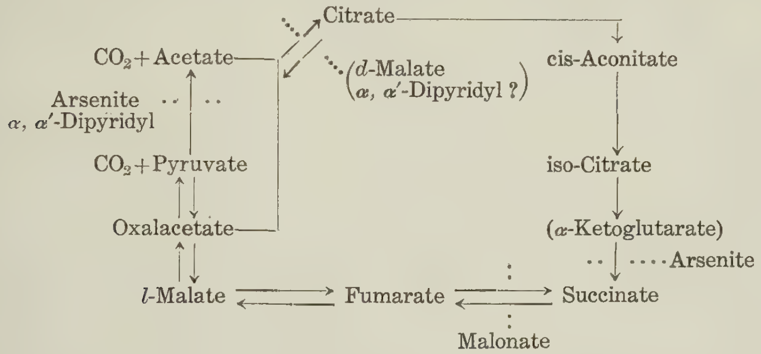
From the above results and some additional evidences, it was probable that if α-ketoglutarate was the member of the tricarboxylic acid cycle in this bacteria, the permeability of the preparation limited the oxidation.

It was found that α, α'-dipyridyl, which had been proved by the authors to inhibit

the anaerobic decomposition of citrate, also inhibited the oxidation of *l*-malate, pyruvate and acetate. It was clearly demonstrated that the reagent inhibited pyruvate oxidase system and at the same time either the activation of acetate or the condensing enzyme.

It was found that a new inhibitor *d*-malate inhibited almost completely the acetate oxidation at rather dilute concentration (M/900), while the oxidation of citrate to acetate was quite stable to d-malate, probably d-malate inhibited the condensing enzyme competing with oxalacetate.

From the inhibition experiments above cited the mechanism of citrate breakdown in *Bact. succinicum* might be summarized as follows:



Dotted lines represent the inhibition by the reagents described.

In aerobic condition, citrate was oxidized exclusively via tricarboxylic acid cycle, because α,α′-dipyridyl did not inhibit the aerobic oxidation of citrate. In anaerobic condition, citrate was decomposed to acetate and oxalacetate by the condensing enzyme(?), some part of the latter compound was reduced to succinate coupled with the oxidation of citrate to succinate via tricarboxylic acid cycle.

There were some evidences that the condensing enzyme was not responsible for the anaerobic breakdown of citrate, since the preparation aged thirty days or more which contained both the activity of acetate oxidation and all enzyme systems belonging to the tricarboxylic acid cycle, did not decompose citrate anaerobically at a detectable rate.

Thus it was concluded that either some cofactor(s?) which was not necessary for the oxidation of acetate played an important role in the breakdown of citrate, or the enzyme different from condensing enzyme was responsible for the direct splitting reaction of citrate.

60. The microbiological determination of amino acids in "Miso". Gakuzo Tamura, Jiro Kirimura, Haruki Hara and Kei-ichiro Sugimura (Journ. Agr. Chem. Soc. Japan **26**, 483, 1952)

The authors determined the amino acid composition of some typical kinds of "Miso" in Japan, namely "Hatcho Miso", "Sendai Miso" and "Edo Miso". They are different in ratio of raw materials and period of brewing, and their amino acids were determined by microbiological method in their following states.

a. A fraction soluble in hot water.

b. Hydrolysate of the whole material.

c. The same as a. (Precipitate formed by tungstic acid or trichloroacetic acid being removed)

d. Hydrolysate of c.

By comparing amino acid composition of "Miso" with that of its raw material, we found that decomposition of essential amino acids during brewing is slight.

From the amino acid composition of a, b, c, d, we discussed the ratio of amino acid liberation during brewing and assumed that "Miso" contains peptide-like substances composed of glutamic and aspartic acid.

61. The microbiological determination of amino acids by lactic acid bacteria. Part 1. Amino acid requirements of lactic acid bacteria and standard curves for amino acids assay. Gakuzo TAMURA, Toshinao TSUNODA, Jiro KIRIMURA and Shigeru MIYAZAWA (Journ. Agr. Chem. Soc. Japan **26**, 464, 1952)

The authors have examined the composition of the basal medium as well as its culture conditions for the microbiological determination of amino acids by lactic acid bacteria.

The authors have studied the amino acid requirement of *Lact. arabinosus*, *Lact. fermenti*, *Leuc. mesenteroides* and *Str. faecalis* in two different conditions ("heavy" and "light" inoculum).

The authors drew the standard curves for 17 kinds of amino acid, improved the composition of basal medium and culture conditions, and determined its assay range.

62. The microbiological determination of amino acids by lactic acid bacteria. Part 2. The determination of amino acids in casein. Gakuzo TAMURA, Toshinao TSUNODA, Jiro KIRIMURA and Shigeru MIYAZAWA (Journ. Agr. Chem. Soc. Japan **26**, 474, 1952)

Resting on the basis of the reported conditions, the authors made a quantitative determination of the amino acids in casein, examined the recovery and reproducibility of assay value and compared our results with other data so far published.

63. The microbiological determination of amino acids in various rice proteins and "Sake". Gakuzo TAMURA, Toshinao TSUNODA, Jiro KIRIMURA and Shigeru MIYAZAWA (Journ. Agr. Chem. Soc. Japan **26**, 479, 1952)

The author have determined the amino acid compositions of rice proteins and "Sake" by microbiological assay.

The authors have determined the amino acid compositions of glutelin, globulin, albumin and prolamin contained in rice protein (Tab. 1), discussed the difference among them and compared the obtained results with other data which have been published already and with the composition of whole egg and whole wheat.

Table 1. Amino acid composition of rice proteins* (%)

Amino acid (Strain)**	Glutelin	Globulin	Albumin	Prolamin
Aspartic acid (M)***	8.0	2.2	7.3	5.1
Arginine (M)	8.4	14.7	5.4	6.7
Cystine (M)	0.7	1.3	2.8	0.6
Glutamic acid (S)	20.8	21.1	17.5	30.2
Glycine (M)	4.6	5.1	4.4	3.8

Histidine (F)	2.9	0.6	2.2	1.0
Isoleucine (A)	5.0	1.6	5.9	4.4
Leucine (A)	9.3	5.9	9.8	10.9
Lysine (M)	2.9	0.8	6.7	0.5
Methionine (M)***	1.3	4.8	1.9	3.4
Phenylalanine (A)	7 .3	3.7	3.9	5.9
Proline (M)	3.8	5.5	5.9	6.3
Serine (M)	6.2	7.0	6.9	6.9
Threonine (M)	3.0	1.6	3.3	1.6
Tryptophane (A)	1.2	1.0	1.4	1.0
Tyrosine (M)	2.7	6.1	3.5	8.3
Valine (A)	8.1	4.3	7.8	7.1

*: N = 16.0 corrected.
**: A, *L. arabinosus*; F, *L. fermenti*; M, *L. mesenteroides*; S, *S. faecalis*.
***: D-activity corrected.

The authors have determined the amino acid content of both hydrolyzed and non-hydrolyzed "Sake" and proved the existence of isoleucine, methionine and threonine in "Sake". The existence of such amino acid as glutamic acid, aspartic acid, glycine and others, which increased when "Sake" was hydrolysed, have led us to a conclusion that there must be peptide or similar substances composed of these amino acids.

The authors have made a comparative study of the amino acid composition of rice protein and "Sake".

64. The microbiological determination of amino acids in "Shôyu". Toshinao Tsunoda, Zentaro Ishizuka, Shigeru Miyazawa and Gakuzo Tamura (Journ. Agr. Chem. Soc. Japan **26**, 477, 1952)

The authors have determined amino acid content of "Shôyu" (Soy Sauce) by microbiological assay.

The presence of arginine, glycine, histidine, serine, threonine, valine and phenylalanine in "Shôyu" were found for the first time and determined by the present study.

The amount of glutamic acid, aspartic acid and glycine after hydrolysis is twice as much that of the intact non-hydrolyzed "Shôyu". This led us to a conclusion that there must be the existence of peptides composed of the above mentioned amino acids.

65. Studies on the odorous constituents of "Saké". Part 1. The detection of volatile acids and esters by the method of paper chromatography. Ginzo Yamamoto, Shigezo Kano and Seinosuke Sugama (Journ. Agr. Chem. Soc. Japan **27**, 3, 1953)

Volatile acids and esters as the odorous constituents of "Saké" have been investigated by paper chromatography. Formic, lactic, acetic and isovaleric were identified as free acids and as components of esters. Caproic and caprylic were also identified as free acids, but they were not detected as components of esters as separated spots on the developed chromatograms.

66. Microbial degumming of fibers. Part 9. Adaptive formation of pectic

enzymes by *Aspergillus niger*. (I). Toshinobu ASAI and Hiûga SAITO (Journ. Agr. Chem. Soc. Japan **26**, 381, 1952)

In the submerged culture of *Aspergillus niger* with *Czapek*-peptone medium, the formation curves of exo-enzymes responding to the presence of different carbon sources are analyzed. As carbon source, glucose, pectin, pectic acid and starch are metabolized in the decreasing order in rapidity. Pectin and pectic acid go into the oxalate formation more slowly than starch and glucose, and homologous two carbon sources are almost similar one another in the effect on the formation of each enzyme in either case. Tendency towards adaptive formation of each pectic enzyme is decrease in the following order; Pectin-methyesterase > Polygalacturonase (galacturonogenic enzyme) > Depolymeric polygalacturonase ≧ Degumming enzyme (retting activity to ramie fiber) ⋛ Protopectinase (activity on maceration of potato). In the early period of cultivation, every enzyme is activated only in the presence of pectice carbon source, while, towards the end of the fungal growth, the latter three are formed even with the heterologous carbon sources. Polygalacturonase activity measured by reducing groups liberated from pectic acid in the prolonged period of enzyme action (20 hours), shows that this enzyme is highly adaptive, while the activity decreasing the viscosity of pectic acid, provisionally called as depolymeric polygalacturonase, is produced constitutively. Thus it is suggested that two or more enzymes are concerned with the hydrolysis of pectic acid. Degumming enzyme and protopectinase may be essentially the same as depolymeric polygalacturonase at least about their formation curves, but not with galacturonogenic polygalacturonase.

67. Microbial degumming of fibers. Part 10. Adaptive formation of pectic enzymes by *Aspergillus niger*. (II). Toshinobu ASAI, Hiûga SAITO and Asami YAMAOKA (Journ. Agr. Chem. Soc. Japan **26**, 389, 1952)

The previous study concerning with the adaptive formation of pectic enzymes is developed by examining the effects of combined supply of heterologous carbon sources. Expecting the economy and the rapid formation of oxalate which seems to accelerate the degumming action on plant fibers, starch or glucose as the growth promoting main carbon source, which gives rise to rapid oxalate formation, and pectin as the accelerator of adaptive formation of pectic enzymes, are added together in the submerged culture of *Aspergillus niger*. Analyzing the formation curves of pectic enzymes and saccharifying amylase, the results are resumed as follows:

(1) As the main carbon source which should be added to pectin, starch is more favorable than glucose for the production of depolymeric polygalacturonase, degummining enzyme and amylase, but less affect pectin-methylesterase and galacturonogenic polygalacturonase. Since the slower metabolizability of starch may elongate the growth phases, causing the increase in the amount of constitutive enzymes and amylase, while the latter two enzymes are adaptive ones which may be produced relating to the amount of adaptive substrate.

(2) The production of the whole pectic enzymes increases, whereas that of amylase decreases, by the addition of pectin to the culture where the main carbon source, starch, has been almost consumed, in comparison with the mixing at the start, presumably owing to the timely acceleration by the adaptive substrate.

68. Studies on the continuous fermentation. Part I. Fundamental studies of

alcoholic fermentation of molasses. Toshinobu ASAI, Kiyomoto UEDA and Tetsuya KOJIMA (Journ. Agr. Chem. Soc. Japan **26**, 564-569, 1952)

Several fundamental experiments of continuous alcoholic fermentation were carried out.

(1) Three excellent strains of alcohol-yeasts were selected for the fermentation of molasses.

(2) Necessary nitrogen source which results in successful fermentation and yeast growth in molasses was quantitatively examined, in both high and low inoculum size.

(3) The available nitrogen content of denatured Formosan molasses was only about 18% of total nitrogen.

(4) No other supplement besides the nitrogen source was needed for the fermentation of Formosan molasses.

(5) The small fermentor vessels were used for the fermentation equipped with a mechanical stirrer and medium was agitated during the fermentation.

69. Studies on the oxidative bacteria. Part 4. On the production of α-ketoglutaric and pyruvic acids by *Pseudomonas* and *Serratia*. Toshinobu ASAI and Kō AIDA (Journ. Agr. Chem. Soc. Japan **26**, 528-533, 1952)

The authors investigated the fermentation products from glucose by the oxidative bacteria. *Pseudomonas* 33 F and *Serratia marcescens* No. 18 were inoculated in a medium containing ingredients in the following proportions: 100 g of glucose, 1 g of $(NH_4)_2SO_4$, 0.5 g of KH_2PO_4, 0.1 g of $MgSO_4$ $7H_2O$, 25 g of $CaCO_3$ and I^L of distilled water. The culture was incubated at 30° by shaking culture. Both of them first oxidized glucose to 2-ketogluconic acid, which was isolated and identified as K-salt (mp. 152°) and chinoxalin derivative (mp. 200°). When fermentation was allowed to proceed further, 2-ketogluconic acid was decreased and α-ketoglutaric acid was produced in a large quantity, which was isolated as Ca-salt and free acid, and identified by elementary analysis as 2,4-dinitrophenylhydrazone (mp. 222°). The acetoin was negative. Pyruvic acid was also isolated and identified from the culture fluid of *Pseudomonas* 33 F. By the paper chromatography of keto acids, this was also confirmed.

At 7.64% glucose concentration, *Pseudomonas* 33 F and *Serratia marcescens* No. 18 produced 1384 mg and 1899 mg of α-ketoglutaric acid per 100 cc of culture fluid, respectively.

Among the five strains of *Pseudomonas* and thirteen strains of *Serratia marcescens*, *Serratia marcescens* No. 18 was most superior in its producing ability of α-ketoglutaric acid.

70. Studies on the oxidative bacteria. Part 5. On the production of 2-ketogalactonic acid from galactose. Toshinobu ASAI, Kō AIDA and Yasuhiro UENO (Journ. Agr. Chem. Soc. Japan **26**, 625-630, 1952)

T. TAKAHASHI and T. ASAI already reported that *Gluconobacter* nov. sp. produced galactonic and comenic acid from galactose. In this experiment we investigated the fermentation products of galactose by the oxidative bacteria by shaking culture, and the formation of 2-ketogalactonic acid via galactonic acid was newly confirmed.

The oxidation of galactose by these oxidative bacteria could be classified into

three types by the curves of reducing power: (1) reducing power decreased rapidly and increased again; (2) reducing power decreased and did not increased again; (3) reducing power decreased and after some time increased again. In the case of type I, at the first stage galactose was oxidized rapidly to galactonic acid, and then, galactonic acid was oxidized to 2-ketogalactonic acid. These facts suggested the concerning enzyme system should be different from that of 2-ketogluconic acid formation, in which the acid occurred simultaneously with glucose utilization as had been reported by STUBBS et al (1940). Optimum pH range for the 2-ketogalactonic acid formation was between 4.4 to 5.2, at 6.0-7.0 retarded remarkably and at 8.0 almost inhibited, while the galactonic acid formation proceeded in wide pH range of 3.5 to 8.0. The *Pseudomonas* strains which produced 2-ketogluconic acid from galactose. The identification of 2-ketogalactonic acid was conducted as K-salt, free acid crystal and methyl ester with the authentic specimens of synthesized acid.

The production of reducing substances from the following hexonic and pentonic acids: Ca-d-gluconate, Ca-d-galactonate, K-d-xylonate, Ca-d-mannonate and Ca-l-arabonate, was also studied by the cell suspensions of *Pseudomonas* 33 F. From Ca-d-gluconate, Ca-d-galactonate, K-d-xylonate, reducing substances were produced but a little from Ca-l-arabonate, and not from Ca-d-mannonate.

71. Studies on the oxidative macteria. Part 6. On the production of α-ketoglutaric acid by *B. megotherium* and *B. Natto*. Toshinobu ASAI, Kō AIDA and Zenziro SUGISAKI (Journ. Agr. Chem. Soc. Japan **27**, 129-134, 1953)

The present investigation has been carried out with the object of selecting the α-ketoglutaric acid producing bacteria. Among the strains of following aerobic bacteria: *B. natto* 1, 2, *B. megatherium saufelice*, *B. megatherium fuscus*, *B. coli*, *B. subtilis* HANNOVER, *B. mesentericus vulgatus* PIORKOWSKY, *Bact. succinicum*, the authers have found that *B. megatherium* DE BARY, *B. natte* I and *Bct. succinicum* produce substantial yields of α-ketogulutaaic and pyruvic acids from glucose.

Examination of the fermentation products was carried out by the paper chromatographic method using the solvent of n-buthanol, formic acid, water (4:1.5:1). In the case of *B. Natto* I, five spots on the paper chromatogram showed R_f values of 0.23-0.24, 0.35-0.37, 0.50-0.52, 0.63-0.65, 0.73-0.75, which represent the positions coincided with the authentic citric, malic, α-ketoglutaric, lactic and pyruvic acids, α-ketoglutaric and pyruvic acids were separated and identified by elementary analysis and melting points of 2,4-dinitrophenylhydrazones.

From 8.64% glucose medium *B. megatherium* DE BARY and *B. natte* I produced 1781 mg and 1262 mg of α-ketoglutaric acid; 335 mg and 344 mg of pyruvic acid per 100 cc of culture fluid, respectively.

72. On the production of fumaric acid by the species of *Roizepus*. Kō AIDA (Journ. Ferment. Ass. **10**, 229-237, 1952)

The author isolated the fumaric acid producing strain of *Rhizopus* species, Rh. B-1, which gave the following yields of fumaric acid at the best conditions, 56.67% in surface culture, 50.4% in shake culture and 38.2% in aerating culture on the basis of sugar consumed.

It was found that starch gave the highest yield of fumaric acid production among the following carbon sources tested, injection glucose, technical glucose,

starch, dextrine and sweet potato flower. But the fermentation was slower than that of glucose. The optimum concentration of glucose was 5-15%. Higher concentration was impractical, because the precipitation of calcium fumarate inhibits the fermentation and incubation time was prolonged.

Eleven kinds of nitrogenous material (peptone, ammonium sulphate, ammonium nitrate, ammonium chloride, urea, sodium nitrate, sodium nitrite, d-aspartic acid, d-glutamic acid, glycocoll) were compared as a nitrogen source, and ammonium sulphate, ammonium nitrate, d-aspartic acid and peptone served as satisfactory sources of nitrogen, sodium nitrate and sodium nitrite failed to produce growth. Optimum concentration of nitrogen source in shake culture was 0.4%.

The influence of heavy metal ions; iron, zinc, copper and manganese, on the fumaric acid production was tested. The addition of zinc caused an increase in the weight of mycelium during the early part of the fermentation, but the weight of mycelium at the end of the fermentation was a little smaller than that of the control. The amount and yield of fumaric acid production was lowered. On the contrary, iron and copper stimulated the production of fumaric acid.

In shake culture, pH change, production of fumaric acid, alcohol and volitile acid, weight of mycelium and nitrogen content of mycelium during the fermentation were studied.

73. Studies on the submerged amylase production. Part 2. Selection of black aspergilli. Yasuji Minoda (Journ. Agr. Chem. Soc. Japan **26**, 128, 1952)

Among one hundred and twenty-three strains of black aspergilli, several superior strains for the submerged amylase production were selected, viz., *Asp. Usamii* R-15-1219, R-15-3821, *Asp. Inuii* var. R-3-4812, *Asp. awamori* var. *fumeus* 6321, *Asp. awamori* var. R-2a-6422, *Asp. awamori* K-5-1032, K-5-0613, K-7-0224, K-5-1714, K-5-1211 and *Asp. aureus* var. *acidus* R-1c-4831.

The saccarifying activity of the *Asp. Usamii* group (*Asp. Usamii*) was high, but its dextrinizing activity was very low. The *Asp. awamori* group (*Asp. Inuii*, *Asp. awamori*, *Asp. awamori* var. *fumeus* and *Asp. aureus*) had high dextrinizing activity, while their saccharifying activity was lower than that of the *Asp. Usamii* Group. *Asp. Nakazawai*, *Asp. awamori* var. *piceus*, *Asp. awamori* var. *fuscus*, and *Asp. Saitoi* were low in both activities.

When used the *Asp. Usamii* Group as the convertion agent, the fermentation efficiency was high, but the time required for the complete fermentation was long. While by the *Asp. awamori* Group it was short, but the fermentation efficiency was somewhat lower.

Then the mixed inoculation for the preparation of submerged amylase culture was examined. In the case of *Asp. awamori* and *Asp. oryzae* the saccharification degree and fermentation efficiency were superior to those of each individual culture. On the contrary the *Asp. Usamii* was not suitable for the mixed method.

74. Studies on the submerged amylase production. Part 3. Selection of industrial culture medium and the chemical changes involved in the submerged amylase production. Yasuji Minoda (Journ. Agr. Chem. Soc. Japan **26**, 173, 1952)

In this paper, the experiments were conducted to find the suitable industrial media for the submerged amylase production, using three types of the *Aspergillus*

group, viz., *Asp. oryzae* (*A. oryzae* 0-9-5), *Asp. awamori* (NP-2) and *Asp. Usamii* (*Asp. Usamii* 1219) which were ascertained to be superior amylase-producing strains in the previous report. The C:N ratio in organic sources was significant. When this ratio was high, the cultures was inclined to be acidic. 3% of rice bran (or wheat bran) and 1-1.5% of Sweet potato powder were adequate as organic source. Addition of $NaNO_3$ as inorganic nitrogen source raised the amylase-activity, but addition of 0.1% of it was sufficient. Other inorganic nitrogen source as $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$ and NH_4NO_3 and urea had the same effect.

The addition of mineral salts such as magnesium sulphate, potassium sulphate, monopottasium phosphate and disodium phosphate gave no remarkable influences on the amylase activity. Phosphate seemed to have some effect, but on the whole, rice bran, sweet potato powder and service water were rich enough in these mineral components. Then it was found that the culture medium consisting of 3% rice bran, 1% of sweet potato powder, 0.1% $NaNO_3$ and 0.2% $CaCO_3$ was adequate for the amylase production in all cases of strains.

The chemical changes involved in the submerged amylase production of these three types of *Aspergilli* were also investigated. Amylase activity rose rapidly, as soon as the autolyse took place and NH_3-N in culture fluid increased. Activity reached the maximum in 65-72 hours.

75. Studies on the submerged amylase production. Part 4. The specific amylase properties owing to the enlarged saccharifying powder of *Asp. awamori* var. *fumeus* 6321 and its application to the alcoholic fermentation. Toshinobu Asai and Yasuji Minoda (Journ. Agr. Chem. Soc. Japan **26**, 190, 1952)

Laboratory experiment on the production of alcohol with submerged amylase of either *Asp. oryzae* 0-9-5 or *Asp. Usamii* 1219 was conducted and the fermentation efficiency of each case was 79.4% and 88.8% respectively, while the time required for the complete fermentation was very long in the latter viz., 137 hours. After the fermentation residual dextrin was large in quantity in the case of *Asp. oryzae*, so we selected strains which possess enlarged saccharifying power and obtained *Asp. awamori* var. *fumeus* 6321 as the superior strain to *Asp. Usamii* 1219. In fact, the experimental data of this strain as the saccharifying agent gave 92.4% of fermentation efficiency and 72 hours for the complete fermentation. Several experiments were also conducted about the effect of successive addition of submerged amylase of this strain to prolong and enlarge the enzymation, but only a little positive effect was observed.

In the course of enzymation, firstly the submerged amylase of *Asp. oryzae* 0-9-5 (the strong α-amylase producer) was added with the yeast inoculum and then the submerged amylase of *Asp. awamori* var. *fumeus*, 6321 (the strong glucogenic enzyme producer) was added after 20 hours intervals, and these mash were set going to complete alcoholic fermentation; where the fermentation efficiency was not promoted in comparison with the case using *Asp. awamori* var. *fumeus* 6321 alone as the saccharifying agent.

The amylase of *Asp. awamori* var. *fumeus* 6321 belongs to "awamori" type according to Okazaki's investigation while this strain showed the property of "Usamii" type when it was cultured in submerged state on the acidic side. The authors pointed out that the formation of amylase components was affected by the pH change in

cultural environment.

76. Studies on the amylase production by submerged culture. Part 5. On the limit-dextrin hydrolysing enzyme. Yasuji MINODA, Toshinori KIKUCHI and Toshinobu ASAI (Journ. Agr. Chem. Soc. Japan **27**, 91, 1953)

In the last stage of alcohol fermentation with starchy materials, it is important to convert residual dextrin, which remains in the mash and is not easily hydrolyzed into fermentable sugar, in order to reduce the length of the fermentation period and to increase the fermentation efficiency. So the authors got residual dextrin from potato starch with mold amylase and studied to obtain submerged mold culture containing the enzyme which can powerfully convert that dextrin. And then several properties of this enzyme were investigated.

(1) *Asp. awamori* var. *fumeus* 6321 which was selected in the previous study was also superior in this case and its superiority for alcohol fermentation was confirmed.

(2) This enzyme was hardly destroyed by treatment, at pH 2.5, 37°, 30 min., when mold culture fluids of *Asp. awamori* type strains were used. But in the case of *Asp. oryzae* and *Rhizopus* the destruction was extreme.

(3) When it is treated at 55°, 15 min., this enzyme was not much destroyed at pH 4.8, but it was destroyed considerably at pH 3.5 and the destruction was extreme at pH 7.0

(4) The optimal pH of this enzyme reaction was about 4.8 in 1 hour of reaction period, but in the case of amylase it was about 5.2 and maltase about 4.0.

(5) The authors assumed through the following facts that this residual dextrin is not hydrolyzed by single enzyme.

(a) In 1 hour of reaction period the optimal pH is about in 4.8 but in 3 hours it is about 4.0.

(b) In acid and heat treatment the different results are obtained between different mold strains.

Table 1. Limit-dextrin hydrolyzing power of attacked (55°C, 15 min. pH 7.0, 4.8, 3.5) mold culture fluid.

		Reaction time		
Mold strain	pH	1 hr. Enzyme activity retained %	3 hrs. Enzyme activity retained %	24 hrs. Enzyme activity retained %
0–9–5	3.5	5.9	10.5	
	4.8	0	0.3	
	7.0		23.7	
	Not-attacked			
1714	3.5	35.4	58.7	78.5
	4.8	57.9	64.8	92.0
	7.0	24.3	32.4	57.5
	Not-attacked	75.4		
6321	3.5	100	74.3	90.2
	4.8	32.2	99.1	99.8
	7.0		49.2	80.0
	Not-attacked	15.8		
Rhiz. Jav. var. *Kawasakiensis*	3.5	46.7	0.1	
	4.8		39.5	57.7
	7.0			
	Not-attacked			

(c) The reducing power decreases during mold enzyme is reacting as shown in Table 1. This fact suggests the existence of some synthetic enzyme.

(d) Ratios of conversion power to hydrolyzing power changes mold strain differs as shown in Table 2.

Table 2. Comparison of hydrolyzing power* and conversion power** of various mold culture fluids

Mold strain	(A) Hydrolysing power (%)	(B) Conversion power (%)	Maltase activity	(B)/(A)
0-9-5	4.3	5.5	5.6	1.28
1219	7.0	10.4	27.0	1.49
6321	12.9	23.8	26.2	1.85
Rhiz. Javanicus var. *Kawasaki*	7.2	10.4	6.7	1.45

* Limit-dextrin hydrolysing power calculated as reducing value increased.

** Limit-dextrin hydrolysing power calculated as fermentable sugar produced.

77. On the inhibitory action of iron on streptomycin production. IV. Takeshi TAKAHASHI and Hiroaki IWAMOTO (Journ. Antibiotics **5**, 263, 1952)

The authors have reported previously that the inhibitory action of iron on streptomycin production were divided into three types according to the forms of iron compounds.

Type A: Inhibition in the medium to which ferric oxide (anhydrous) was added.

Type B: Inhibition in the medium to which ferrous or ferric sulfate or hydrous ferric oxide sol was added.

Type C: Inhibition in the medium into which iron dissolved from a steel plate and which was not hested after that.

In this paper the differences of the properties of the colloid between type B and C were examined.

The electronmicrographs of the iron colloids and the appearance of their adsorption to the mycerium in the two kinds of media were taken. And their electrophoretic verocity was examined.

It became clear that these two are different in stability, activity and size from each other.

The concentration of ferrous ion in the broth was also determined. But any relation was not found between the quantity of ferrous ion and the inhibition of streptomycin production.

78. On the inhibitory action of iron on streptomycin production. V. Takeshi TAKAHASHI and Hiroaki IWAMOTO (Journ. Antibiotics **5**, 266, 1952)

The inhibitory concentrations of iron for streptomycin production of the several strains isolated in Japan were determined and it was found that they were much the same.

The amount of iron dissolved into WAKSMAN's medium from a steel plate was twice as much as that dissolved into UMEZAWA's soy bean medium. The iron concentration which was inhibitory to production of streptomycin in WAKSMAN's and UMEZAWA's media was found to be the same.

The effects of the constituents of the medium on the dissolution of iron from

a steel plate were studied. It was found that corn steap liquor was a very strong inhibitor for dissolution of iron.

79. On the inhibitory action of iron on streptomycin production. VI. Takeshi TAKAHASHI and Humiko SEKINE (Journ. Antibiotics **6**, 33, 1953)

The quantity of iron which inhibits the chloramphenicol production in submerged culture was studied and it was found that the inhibition curves by $FeSO_4 \cdot 7H_2O$ and test piece are different from each other in glycerin-peptone-Czapec medium as in streptomycin production.

The consumption of inorganic phosphorous of some streptomycin producers is far larger than that of chloramphenicol producers.

The fact that the inhibition of iron in practical chloramphenicol production might be explained by the presence of inorganic phosphate which remains in a quantity enough to work as corrosion inhibitor.

80. Studies on the utilization of the Waldhof fermentor. Part 2. Continuous production of baker's yeast. Koichi YAMADA, Takashi ITO, Yukihiko HANDA and Akira KOYAMA (Journ. Agr. Chem. Soc. Japan **26**, 335, 1952)

A 501 stainless steel Waldhof type fermentor was used for the continuous production of baker's yeast from molasses under various conditions of pH of feed, sugar and nitrogen concentration of feed, and feeding rate, etc. Twenty three series were run and each run was maintained in a stationery state for eight hours, checked every hour. The best result was obtained under the conditions that the sugar concentration of feed was 6.80% (the highest concentration tested), pH of feed was 8.8, the feeding rate was 3.01 and the concentration of yeast in the fermentor was maintained at 12.3 g (wet) per 100ml. The yield of yeast (wet) was 186.8% based on the total sugar and 202.4% based on the sugar used. The activity of the yeast was satisfying compared with the commercial baker's yeast.

These results make it clear that the Waldhof type fermentor is the most suitable fermentor not only for the production of fodder yeast from sulfite waste liquor but also for the production of baker's yeast from molasses.

The superiority of Waldhof type fermentor to normal sperger type one is shown in the following table:

	Normal sparger type	Waldhof type
Sugar conc. of wort.	1.9—2.0%	6.8%
Yeast yield (wet) based on total sugar.	120%	185%
Yeast yield (wet) from 100 kg molasses	60 kg	92 kg
Volume of liqure used per day (10 t fermentor)	0.57 t	2.04 t
Yeast yield (wet) per day (10 t fermentor)	0.34 t	1.88 t
Capacity of fermentor required for 1 t yeast per day	29.5 t	5.3 t
Volume of air required for 1 kg dry yeast	120 m^3	11.3 m^3

81. Fundamental studies on the aerobic fermentation. Part 1. Oxygen absorption rate in shaking cultures. Kōichi YAMADA, Jyōji TAKAHASHI and Nakaji KOJIMA (Journ. Agr. Chem. Soc. Japan **26**, 427, 1952)

The partial pressure of O_2 in a cotton stoppered flask, which contains fermenting broth, must be lower than the partial pressure of O_2 in the atmosphere, owing to the presence of remarkable amount of CO_2. Under stationary conditions, the amount

of O_2 which transfers through the cotton stopper is equal to that of O_2 which transfers through the gas-broth interface. Based on the diffusion theory, the following equation is obtained, on the rate of oxygen transfer.

$$dW/d\theta = K_1(P_a - P_f) = K_2(P_f - P_L) \quad \cdots\cdots (1)$$

hence, dW : gr. mol. of O_2 transfered into medium during $d\theta$

K_1 : diffusion rate coefficient at the cotton stopper (gr. mol. of O_2/min. atm.)

K_2 : diffusion rate coefficient at the gas-medium interface (gr. mol. of O_2/min. atm.)

P_f : partial pressure of O_2 in gas phase in flask (atm.)

P_a : partial pressure of O_2 in atmosphere (atm.)

P_L : partial pressure of O_2 in medium in flask (atm.)

In practical cases, partial pressure of O_2 in medium, P_L, can be assumed to be zero. Therefore, the rate of oxygen transfer into the medium is given by the following equation.

$$dW/d\theta = K_2P_f = K_1K_2P_a/(K_1+K_2) \quad \cdots\cdots (2)$$

This equation (2) indicates that the rate of oxygen supply to the medium depends upon K_1 and K_2, which are determined independently of the existence of micro-organisms in the medium.

Then, in order to know the effects of the changes of conditions—method of shaking, tightness of cotton stopper, constituents of medium and volume of medium in flask, etc.—we have determined the values of K_1 and K_2 in several different cases.

As the results of those experiments, the following facts were known.

The diffusion rate coefficient at gas-medium interface, K_2, depends mainly upon the method of shaking, and the effects of the other conditions—temp. of medium, constituents of medium, volume of medium in flask, etc.—were comparatively small. The values of K_2 obtained by the experiments with the medium of 100ml. of malt extract were, 5.98×10^{-4} on the reciprocal shaker (stroke: 70 m/m; 140 strokes/min.), and 1.5×10^{-4} on the rotary shaker (rotation radius: 25 m/m; 220 r.p.m.)

The effect of tightness of the cotton stopper, temp., etc. on K_1 were much smaller than that of the partial pressure of CO_2 in the shaking flask. The values of K_1 obtained were, 1.395×10^{-4} in the case that the partial pressure of CO_2 was 1, and 3.16×10^{-4} in the case that the partial pressure of N_2 was 1.

From the above results, we can calculate the amount of oxygen supply to the shaking flask which contains fermenting broth, in ordinary cases, the amount of oxygen supply to 100 ml. of medium are 0.7-1.32 mg./min. on the reciprocal shaker and 0.43-0.63 mg./min. on the rotary shaker at 27.5°C.

82. Studies on the thermophilic cellulose-decomposing bacteria. Part 2. On the products and their quantitative relationship by the purified culture. Kōichi YAMADA, Yoshinori FUJIMOTO and Yuzuru FUNADA (Journ. Agr. Chem. Soc. Japan **26**, 323-325, 441-444, 1952)

Qualitative and quantitative analysis of the fermentation products of the purified culture acting on cellulose and dextrose had been made and the fermentation balances set up. The main products identified were carbon dioxide, ethyl alcohol, acetic acid, lactic acid and sometimes butyric alcohol and butyric acid. Showing up to 75% cellulose decomposition and 54% carbon recovery, and producing 11% alcohol, 22% volatic acid, 24% unvolatile acid, more than 60% carbon dioxide, the mutual ratio

of the main products approached to the values of which were demonstrated in case of using dextrose. That is, ethyl alcohol : butyl alcohol = 3.8 : 1, acetic acid : butyric acid = 3.2-3.9 : 1, alcohol : volatile acid = 0.5-0.6 : 1 etc.

During the fermentation, alcohol and volatile acid were considerably vapoured up owing to the high temperature. For instance, when alcohol was added in contaminant culture (48.4 mg/100 cc), 42% volatilized fraction, 52% consumed fraction and 7% residual were detected after 4 days. And the main products after 5 days cellulose fermentation, in which 54% cellulose was digested, showed that the ratio of alcohol in the medium and in the vapour was 11 : 7, and volatile acid in the medium and in the vapour was 15 : 7.

As intermediary products depended on digestion of cellulose and Na-C.M.C., dextrose and cellobiose were detected as osazone by enzymic accumulation.

From these facts it is clear that the hydrolysis and fermentation of cellulose were caused almost momentarily, and some products were transformed into other substances.

83. Studies on the thermophilic cellulose-decomposing bacteria. Part 3. The effect of lignin on the cellulose decomposition and some aspects of the practical value of this fermentation. Kōichi YAMADA, Yoshinori FUJIMOTO and Yuzuru FUNADA (Journ. Agr. Chem. Soc. Japan **26**, 470-473, 1952)

For the purpose of utilizing the cellulose digestion on an industrial scale, the decomposition products of various kinds of woods and the effect of lignin on the fermentation were investigated.

In spite of a powerful attack on cellulose or pentosan, cellulose thermophile could not digest lignin isolated chemically by which the fermentation was not effected.

The main interest from the industrial aspect had been in the obtaining of alcohol. Delignificated woods were fermented up to 97% producing 25-34% organic acid, although ball-milled woods and ground pulp were not decomposed. But the production of alcohol and recovery of carbon were too poor to be applied on an industrial scale.

These results were as follows:

Chlorinated		Not treated	1 time	2 times	4 times
Composition[1]	Cellulose	53.23	80.61	88.31	100
	Pentosane	8.99	13.21	8.50	0
	Lignin	30.56	1.78	0.88	0
Incuvation time (days)		30	8	8	8
Decomposed %		7.9[2]	95.2	97.1	97.2
Products[3] %	alcohol	0	0.5	3.1	0
	Total acid	0	34.4	31.2	25.0
C recovery %[4]		—	31.2	28.1	21.9

1) Chamae-cyparis sp.
2) Extraction by hot water (60°C), and not decomposition by bacteria.
3) Based on wood decomposed.
4) Based on cellulose and pentosan decomposed.

In situ, cellulose thermophile could not attack the tissue decayed by fungi or the ball-milled tissue, so it was considered that cellulose bacteria had no such enzymic action on woods as was shown by wood destroying fungi.

84. Studies on the production of vitamin C by fermentation. Part 1. Preparation of Ca-l-idonate and Ca-d-gluconate from Ca-5-keto-d-gluconate. Masataka YAMAZAKI and Tahei MIKI (Journ. Ferment. Technology **31**, 39, 1953)

In the presence of RANEY Nickel as catalyst, Ca-5-keto-d-gluconate which was prepared from d-glucose by the oxidative fermentation of *Acetobacter suboxydans* 621, could be hydrogenated under atmospheric or high pressure into the mixture of Ca-l-idonate and Ca-d-gluconate.

l-Idonic acid was separated as dibenzal-l-idonic acid and identified as its brucin salt. After removing benzaldehyde with ether the filtrate was neutralized with $CaCO_3$ and $Ca(OH)_2$, and Ca-d-gluconate was obtained. The total yield of both acids was about 90-93% (l-idonic acid 47%, d-gluconic acid 43%). Lastly, it was observed that, even in high pressure reduction, the mixture was not epimerized to Ca-l-gulonate and Ca-d-mannonate.

The details of temperature and time used for the reduction of Ca-5-keto-d-gluconate was observed.

85. Studies on the production of vitamin C by fermentation. Part 2. Isolation of 2-keto-acid producing oxidative bacteria and chemical separation of 2-keto-acids. Masataka YAMAZAKI (Journ. Ferment. Technology **31**, 86, 1953)

Pseudomonas fluorescens sp. producing a large amount of 2-keto-acids from Ca-l-idonate, Ca-d-gluconate and their mixtures was isolated from the soil. Optimum conditions for the oxidation with cell suspension were proved to be pH 5.0 and about 0.9 g of wet cells per 3 g substrate in 70 c.c. of the medium used. The maximum yield of 2-keto-acids was about 70% on the basis of the mixture consisting of Ca-l-idonate and Ca-d-gluconate. 2-Keto-gulonic acid and 2-keto-gluconic acid was separated and identified as Na- and K-salts respectively.

86. Studies on the production of vitamin C by fermentation. Part 3. Oxidation of Ca-l-idonate and Ca-d-gluconate mixture by *Pseudomonas* sp. in shaking culture. Masataka YAMAZAKI (Journ. Ferment. Technology **31**, 126, 1953)

In order to promote the bacterial growth and its oxidative activity, the initial pH of 1% yeast-extract-medium was adjusted to 6.7 and after 3 days' preincubation at 24°C the pH was reduced to 5.0 by the addition of substrate (mixture of Ca-l-idonate and Ca-d-gluconate, 5 g per 100 c.c. medium) and then the fermentation was carried out, adjusting the pH value to 5.0 every 24 hrs. In this case high yield of 2-keto-hexonic acid was obtained, i.e., after 7 days it reached 40.2% on the basis of the substrate, and mainly accumulated 2-keto-gluconic acid, while after a 11 days' incubation the main product was 2-keto-l-gulonic acid. Accordingly, the former was more easily formed and decomposed than the latter.

In the second experiment, the pre-incubation of the bacteria in d-glucose-yeast-extract medium containing a inorganic source was conducted during 40 hrs. In this case a vigorous respiration occurred, and 2-keto-hexonic acid was hardly accumulated. Then the substrate was added after adjustment of pH to 5.0 and the successive fermentation was set going under the above described pH regulation.

After 40 hrs., the production of 2-keto-hexonic acid reached 51.5% on the basis of the substrate, and it comprised a larger amount of 2-keto-l-gulonic acid than 2-keto-d-gluconic acid.

Pseudomonas fluorescens liquefaciens (Bacterial Museum, KRAL), *Pseudomonas fluorescens* 33 F (isolated by YOKOZAWA in 1950) and *Pseudomonas fluorescens* Y (isolated by the author in 1951) were compared in their activity producing 2-keto-hexonic acid and it was shown that the last strain was the most active, i.e., the yield was 50.3 2-keto-hexonic acid on the basis of the substrate, after a 3 days' incubation in the above described fermentation process.

87. Studies on the production of vitamin C by fermentation. Part 4. Oxidation of Ca-l-idonate and Ca-d-gluconate by *Pseudomonas* in shaking culture. Masataka YAMAZAKI (Journ. Ferment. Technology **31**, 230, 1953)

Such low concentration of $(NH_4)_2SO_4$ as 0.1% and the regulation in acidic side (pH 5.0) after a vigorous multiplication of the cells was preferable for 2-keto-hexonic acid formation.

Oxalic and acetic acids were tested for the effect of buffer action. The yield of 2-keto-hexonic acid was 46.2% with the former and 47.% with the latter. However, the acetic acid was favorable in facilities of observing the multiplication of bacterial cells and separating crystalline 2-keto-hexonic acid.

When the concentration of inorganic salts was increased to six times, the formation of 2-keto-hexonic acid was lowered by 7-10% than the usual. Thus the previously decided concentrations, i.e., 0.05 g/100 cc of KH_2PO_4 and 0.01 g/100 cc of $MgSO_4 \cdot 7H_2O$, was preferable.

The following N-sources were found to be suitable for the formation of 2-keto-hexonic acid as high as 6%, and the yields of crystalline Na-2-keto-l-gulonate were as follows:

N-sources	Yield of Na-2-keto-l-gulonate (% to the substrate)
$(NH_4)_2HPO_4$	32.2
$C_2H_3O_2NH_4$	40.4
$CO(NH_2)_2$	33.0

88. Studies on pH of fish muscle. Part 1. Variation in pH of fresh albacore muscle on the locality. Masao FUJIMAKI, Toshiharu KAWABATA, Keishi AMANO and Fumiko TOMIYA (Bull. Jap. Soc. Sci. Fish. **18**, 124-132, 1952)

Using glass electrode pH meter, studies on pH of fish muscle have been carried out to know the reliability of pH test employed in freshness examination of fish meat and the factors affecting on its test. Marked differences were observed in pH of the muscles of albacore (*Germo alalunga*) tested immediately after catch, depending on the locality of muscle. The pH of the muscles in the head and those around the pectoral fin were found unsuitable to be used as indices of the quality of the fish since they were not always in parallel with the freshness of trunk muscles. The variation in pH of fresh muscle was related with the amount of lactic acid and ammonia, and the reason for high pH value in the orbital muscle and buccinator was found mainly due to the low content of this acid. It should be noted that the dorsal muscles of albacore tested immediately after catch in summer, particularly when the body was in rigor mortis, have been found to have volatile basic nitrogen contents above 25 mg%, contrasting with the figures for winter albacore meats which were so low as 10 mg% as shown in this experiment. This phenomenon seems to have some influence on pH of the fish meat.

89. Studies on the vitamin C in canned mandarine orange. (I) On the true vitamin C value in canned mandarine orange. (II) On the vitamin C enriched-canned mandarine orange. Masao FUJIMAKI, Satoshi ODAGIRI and Choten INAGAKI (Journ. Jap. Soc. Food and Nutrition **5**, 187-192, 1952)

Vitamin C values in canned mandarine orange, determined by 2,6-dichlorphenol-indophenol method, seemed to be erroneous on account of the formation of reducing substances in the process of manufacture, but were found to be true values, in good agreement with the results obtained by 2,4-dinitrophenyl hydrazine method, polarography and animal test. Experiments on the retention of vitamin C in vitamin C enriched canned mandarine orange were carried out.

90. Chemical studies on the preparation of peptones. II. Experiments on the action of pancreatin. Rinjiro SASAKI and Makoto KAMIYA (Jap. Journ. Zootech. Sci. **23**, 110-113, 1952)

Several experiments to determine the optimum condition of the action of pancreatin, and to make peptone products from milk casein, blood fibrin and serum protein, have been performed.

The same as the first report on the action of pepsin (*Ibid.* **20** (4), 97-102, 1949), it was recognized that the formol titration method was able to be applied to compare the degrees of digestion though necessary to provide the titration standard for each protein hydrolysate to make clear the end point. By this method it was decided that the optimum temperature was about 50°C for all samples and the optimum reaction pH 7.6-8.2 for casein, pH 7.2-7.4 for fibrin and pH 7.8 for serum protein in the course of digestion.

The reaction of protein solution changes gradually to acid side till about 20 hours after the preparation of solution, so it must be adjusted at times with the optimum one by adding buffer solution in order to digest these substrates for long time.

As the results obtained, casein is easily digested, fibrin with difficulty digested (but its digestibility inireases very remarkably along with the digestion period) and serum protein scarcely digested. In serum protein antitrypsin will exist.

The highest yield of peptone in casein hydrolysates is 39% of total nitrogen after 12 hours digestion, and that in fibrin hydrolysates 37% after 48 hours. Residual nitrogen (polypeptides and amino acids) shows the highest value after 48 hours digestion, 42% of total nitrogen for casein and fibrin.

After all in the practical purpose, it will be profitable to digest for 48 hours.

91. On the phosphatase in cow's and goat's milk. I. Qualitative test. Tomokichi TSUGO (Jap. Journ. Zootech. Sci. **23**, 68-69, 1952)

By means of the modified form of SCHARER's short field test, content of phosphatases of cow's milk was compared with that of goat's milk, and the effect of heat on the one compared qualitatively with that on the other.

It was found that the phosphatase content of goat's milk was less than that of cow's milk and readily destroyed by heat. For example, at a standard temperature of 62°C of pasteurization, the time of heating in which the phosphatase reaction of cow's milk was turned to negative was 25 minutes, while in goat's milk it was 10 minutes. It seemed to be unable to distinguish the heating period of 25 minutes

from that of 30 minutes at a heating temperature of 62°C of cow's milk by this qualitative test.

93. On the phosphatase in cow's and goat's milk. II. Quantitative test. Tomokichi TSUGO (Jap. Journ. Zootech. Sci. **23**, 70-72, 1952)

Contents of phosphatases of cow's milk were compared with that of goat's, and the effect of heat on the one compared quantitatively with that of the other by means of the modified form of SCHARER's laboratory method using PULFRICH's photometer.

(1) In the raw milk, contents of phosphatase in cow's milk were 400-550 of SCHARER's units, while in goat's milk they were 350-400 units. These values had no connection with fat contents of their milk.

(2) The relation between temperature and time of heating in which phosphatases of cow's and goat's milk were turned to be inactivated, was presented. For example, at a standard temperature of 62°C of pasteurization, in cow's milk the time of heating was 30 minutes, while in goat's milk it was 15 minutes.

As a whole, it was found that the phosphatase of goat's milk was destroyed at a lower temperature of 1-2°C and 10-15 minutes faster than in cow's milk.

93. Comparisons between the coagulation and decomposition of milk by rennet and pepsin. Tomokichi TSUGO (Jap. Journ. Zootech. Sci. **23**, 178-180, 1953)

The milk-coagulating action of rennet has been compared with that of pepsin concerning pH and temperature. When pH of milk was high (pH 6.5-6.6), the milk-coagulating power of rennet was much higher than that of pepsin, but when it was comparatively low, the both powers turned out almost the same. On the temperature, the higher it raised to 45°C, the larger coagulating power of rennet became, however, the power of pepsin was maximum at 35°C and was reduced appreciably at 45°C.

The proteolytic action of rennet on the casein of skimmilk has been compared with that of pepsin at 35° and 40°C. It was found that differences between their proteolytic actions in temperature ranging from 35° to 40°C were very little. In the case of rennet, its action was not much varied in the reaction of pH 6.5 to 4.0, but a rapid increase of its action occurred at about pH 2.0. On the other hand, a gradual increase occurred with the lowering of pH in the case of pepsin.

The property of coagulants produced by rennet from skimmilk has been compared with that produced by pepsin concerning the chemical composition. The result showed that the difference was very little.

III. Agricultural Economics

Abstract Nos. 94—95

94. The role of the Hypothec Bank of Japan in the economic development in Japan. Yuzuru ITŌ (Journ. Rural Econ. [Nōgyō Keizai Kenkyū] **24** [3], 19-33, 1953)

The Hypothec Bank of Japan (Nippon Kangyō Ginkō) has been set up in 1897, modelled on the Crédit Foncier de France. This bank was empowered to make loans, redeemable by annual instalments within fifty years, on the security of immovable

property such as paddy fields, upland fields, salt fields, forests and fishering waters and rights (later urban real estates). It was also permitted to make advances without security to local public authorities, co-operatives of peasants and fishering guilds. In order to obtain funds, it was authorized to issue debentures to an amount not exceeding ten times (later fifteen times) its paid-up capital. At that time, Japan had just won the Sino-Japanese War of 1894-95, and was planning the speedy economic development. This bank was established to be the bank specialized on granting long-term loans largely to agriculturalists, small country manufacturers, and especially to the former. It was the huge bank in the Japanese financial world at that time, with its capital of 10 million yen, a-twentieth of the war expenditure for Sino-Japanese War (1894-5). There were only four banks which had more than 10 million capital at that time. In 1912 its issued debenture was 53% of the total issued bank debentures, 28% of total issues (contained corporation debentures). With the passage of year Japanese economy has made a great progress. In that remarkable process of development of Japanese economy, however, this bank declined in its relative importance. In 1928 its issued debenture was about 29% of total issued bank debentures, about 11% of the total issues (contained corporation debentures). See Table 1.

Table 1.

	Total issued bank debentures (A)	Total issued corporation depenture (B)	Total (C)	Issues of Hypothec bank debenture (D)	(D)/(A)	(D)/(C)
	thousand yen	thousand yen	thousand yen	thousand yen	%	%
1912	80,678	73,253	153,931	42,786	53.0	27.8
1928	707,894	1,191,565	1,899,459	205,217	29.0	10.8

Source: Asahi Press. Nippon Keizei Tôkei Sôkan.

Not only its weight in financial world decreased, but this process was not linear. There was negative correlation between its activity and the whole business activity.

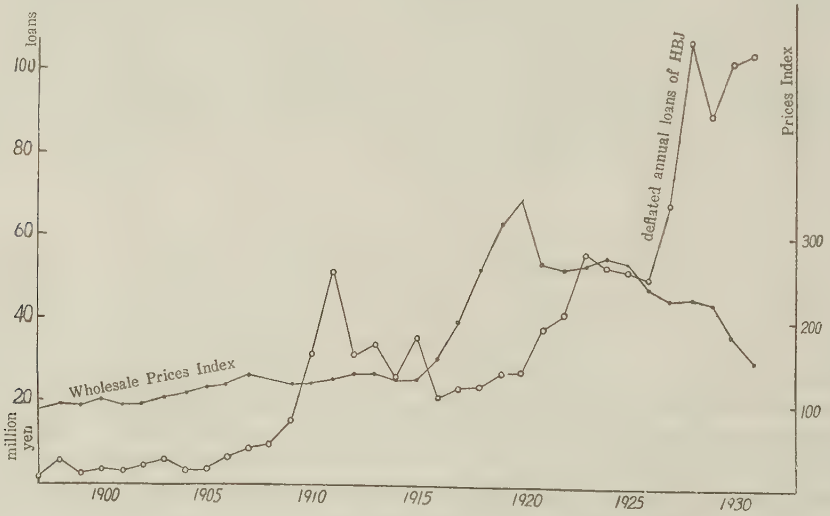


Fig. 1

When the whole business world was voyant, the activities of this bank shrinked and when whole business world panted in depression, then the business of this bank was reversely expanded. The relation is as shown in Fig. 1. In this figure the whole business activities are represented approximately by the Tokyo Wholesale Prices Index of the Bank of Japan, and those of this bank by its annual loans deflated by the above index. (Annual loans are here preferred to loans outstanding, for the former is seemed to represent the annual activity more correctly than the latter).

As this figure shows, this bank declined in its relative importance in the Japanese economy showing the negative correlation with the whole business activity as her economy developed. How shall we explain this fact? The reasons are to be found in the characteristics of its loan activity. It had two attributes. Firstly, this bank made loans on the security of immovable property, and, in this sense, it was a hypothek bank. Secondly it was specialized on granting loans largely to agriculturalists, so it was an agricultural bank. It is the main purpose of this article to explain that these two attributes were the explaining factor of the above fact from the both viewpoints of trend and cyclical fluctuation.

The ages considered are thirty-four years, 1897-1931. Gold standard was established in Japan in 1897, when Hypothec Bank of Japan was established. 1931 was the year when Japan abandoned the gold standard. As they say, Japanese economy is so-called a political economy in which economy is closely connected with political force, and often led by it, but thirty-four years considered seem to be the years during which economy is least connected with political power in the history of Japanese economy.

95. The analysis on the dairy farming in Shizuoka Prefecture. Sōhei MARUTA (Journ. Rural Econ. [Nōgyō Keizai Kenkyū] **24** [3], 44-60, 1953)

Introduction

(1) Problem—It will be asked, first, what types are found out among dairy farm-houses in Shizuoka Prefecture, secondary, why did they take place, thirdly, what results are derived when a structural hypothesis is operated to change in order to verify the second question.

(2) Procedure—We made the field work of a complete enumeration of dairy farm-houses within each village in five counties, and used brief questionaires for our complete enumeration, but detailed ones about their management and individual histories for a few large scale farms.

(3) Method—The numbers of dairies kept at each farm-house were made the control to suppose frequency distributions in order to seek any type among them, and next we sought some kind of differences on the structure within each village and between villages, owing to the distribution types. Then Poisson distribution type was assumed and the shifting of its mean values was regarded as projecting each stage of productive capital accumulation. After productive processes were researched, we supposed the cause of typification as a oligopsonistic market, which was only a hypothesis.

(4) Operation—Following the different methods, we observe what result would take place, if that relationship changes mechanically and historically, to test the oligopsonistic hypothesis.

Facts and Typification

From the distribution of numbers of dairies kept and table 1, we could classify them into two main types, say milk production type and dairy and calf production one, in case in company with dealer one. And about those attributes, we find out a tendency that labor productivity raises with the increase of dairy numbers kept but output decreases halfway. Such a trend is similarly seen either between villages or within a village.

Table 1.

			I	II	III	IV	V
Average number of dairies per farm at each village		(head)	0.43	0.57	0.38	1.00	1.7
Periods		(year)	4	5	15	33	40
Organic composition of capital			.15	.25	.26	.67	.45
Output per day-dairy	village mean	(lit.)	6.3	11.3	12.1	8.1	15.3
	large farm	(lit.)	4.9	12.2	12.1	9.0	18.0
Labor input per dairy	village mean	(hour)	6.5	4.0	3.8	1.5	2.2
	large farm	(hour)	5.0	3.8	2.2	1.0	1.7
Output per labor-hour	village mean	(lit.)	1.1	3.1	3.9	5.4	7.0
	large farm	(lit.)	0.9	3.2	5.6	9.0	13.9

The structure and relationship between those types. It has been so far believed that the economic limiting factor of dairy farming in Japan has been feed, which charged a high land rent. Thus, though we counted up each correlation coefficient between arable land and milk output and the number of dairies kept, it was negligible. Thus when we proceed into the distributing process, the oligopsonistic markets are seen as in table 2 and also the index number of milk price is lower than that of dairy price as table 3 shows.

Table 2.

County	Factory and milkman	Demanded volume
Shuchi	Meiji Iwata Fac.	10.709 kg
	Meiji Misakubo F.	7.174
	Shuchi Milk	2.031
Ogasa	Meiji Fujieda F.	76.642
	Kakegawa Milk	3.207
	Horinouchi Milk	1.467
Kamo	Morinaga Nyugyo Shimoda Fac.	165.011
	Jotomura Nokyo	?
	Shimoda Milk	1.819
	Chikuma Milk	1.090
	Matsuzaki Milk	770
	Ukushi Milk	?
Tagata	Morinaga Mishima Fac.	34.566
	Nittorakuno unlim.	5.408
	Nisshinrennyu j, c	16.754
	Yagisawa Rennyu	11.059
	Fujiya-shokuhin	13.455
	Mishima Milk	2.573
	Ohito Milk	576
	Tobu Milk	5.739
	Kobayashi Milk	3.795
	Miyauchi Milk	2.829
	Ito Milk	?

Table 3.

Year	Dairy (calf)	Milk
1937	100	100
'38	117	133
'39	144	150
'40	192	183
'41	205	189
'42	232	211
'43	262	228
'44	265	283
'45	377	533
'46	18.094	5.205
'47	48.009	17.361
'48	64.540	28.888

Now even if we intend to examine a cost analysis of the milk industry, they never publish their truce balance sheet for tax-dodging and a union policy. Moreover, even if demand and supply equations have been composed to derive a normal milk price to compare it with a real price, structural parametres are made from the observables which has already included the oligopsonistic character, and therefore its solution contains necessarily such a character. Thus we fall into an argument in a circle, and proceed an indirect verification of necessity.

Verification

Accordingly, making use of the different method, unless there is a oligopsonistic market, such a price is to be reduced, therefore no type takes place, thus farm milk production will be developed. Now we take a imaginable experiments. First, if we seek geographically a region where monopsonistic relationship is changed to decrease, in Kamo County, it is found out for every share at Morinaga-nyugyo joint-stock company to have been divided into three parts, say old capitalists, workers in the factory and dairy farm-houses. Since thus peasants could get the power determining the milk price, milk production was mostly raising. Secondary, going back in the historical direction, it is found out at Tagata County they have resolved the oligopsonization and have recovered free competition among buyers whose price policy has been reduced, and consequently dairy production or dealer types disappeared.

Thus we could verify that it is of oligopsonistic price formation to raise the preceding types of dairy farm-houses.

IV. Agricultural Engineering

Abstract Nos. 96—108

96. Experiment on duty of water in irrigation of upland-rice. Masuji AKIBA (Rept. Kanagawa Prefecture 1952)

The author established two non-irrigation plots, two 30 mm irrigation plots, two

50 mm irrigation plots and two plots which are irrigated at any time, dividing upland fields of 480 m^2 wide into 12 plots. The farm land is located on a plateau of Kantoh loam at Yamato, Kanagawa Prefecture. Thus he investigated the relation between the quantity of irrigation water of every plots and its production, and the fluctuation of moisture content of soil.

Irrigation was practised five times ranging from 9 August to 12 September, 1951.

The relation between production per *tsubo* of every plot and amount of irrigation water is as follows:

	plot name	total weight	weight of unhulled rice	weight of stalks	weight of unhulled rice/total wt.	weight of stalks/ total wt.
		kg	kg	kg	%	%
50mm plot......	A	2.63	1.05	1.58	40.0	60.0
	B	2.10	0.86	1.24	41.0	59.0
	average	2.37	0.96	1.41	40.5	59.5
30mm plot......	C	1.99	0.85	1.14	42.7	57.3
	D	1.81	0.75	1.06	41.4	58.6
	average	1.90	0.80	1.10	42.1	57.9
nonirrigation	E	1.64	0.79	0.85	48.1	51.9
plot............	F	1.80	0.75	1.05	41.7	58.3
	average	1.72	0.77	0.95	44.9	55.1

From the above result the following conclusion is reached:

(1) As for production per *tsubo* (weight of unhulled rice), 50 mm plot is 125% of non-irrigation plot, and 30 mm plot 104% of the same; in other words, 50 mm plot and non-irrigation plot had the maximum and the minimum production respectively.

The total rainfall during the irrigation season from 19 July to 12 September was 340.8 mm.

(2) The total irrigation water in 50 mm plot reached 590 mm (rainfall + irrigation water); this method of irrigation was found to be most effective from the above result, showing that 50 mm irrigation every week was most favorable. (The amount of rainfall may be deducted from the quantity of irrigation water).

(3) The percentages of the weight of unhulled rice to the total weight are in the order of non-irrigation plot, 30 mm plot and 50 mm plot, and those of the weight of stalks to the total weight are contrary to the above. In other words, the growth of stalks is delayed very much in case where irrigation water is short.

The author investigated on the fluctuation of moisture content of soil during the irrigation season for about a month ranging from 2 August to 3 September, the result of which is as follows:

Both 30 mm plot and 50 mm plot had a moisture ratio of about 140% at the depth of 70 cm under the ground, which remained nearly constant throughout the season, and a remarkable fluctuation was shown at the depth of 30-50 cm where root zones were spreading widely.

It was about 80% on an average at the depth of 30 cm, and about 100% at the depth of 50 cm in 50 mm plots, and it was about 70% at the depth of 30 cm, and 90-100% at the depth of 50 cm in 30 mm plots. At the depth of 10-20 cm about 50-60% in 50 mm plots and 40-50% in 30 mm plots were observed respectively.

As the soil is likely to get dry from the above result in case when root zones lie

lower than 20 cm, and 30 mm irrigation is practised at the intervals of 7 days, times of irrigation should be increased, and 50 mm irrigation every 7 days is more advisable.

97. Investigation of percolation loss of earth canal. Masuji AKIBA (Rept. Kanagawa Prefecture 1952)

The loss of discharge while irrigation water is being conveyed by an earth canal occupies an important part among those factors for determining duty of water of upland irrigation.

In this investigation the author wanted to clarify the relation between the losses of various discharges and such items as age, condition and quantity of flow of canal by means of a newly cut earth canal 425 m. long and an old canal 2,661 m. long.

The experiment was practised on a plateau of Kantoh loam at Sagamihara, Kanagawa Prefecture, in September and December in 1951, and triangular weirs were established for the discharge measurement.

Thus the following results were obtained:

(1) In case when irrigation water was conveyed by the earth canal and the discharge reached more than a certain amount, the loss increased by a straight line with the increase of the discharge.

(2) In this case the percentage of the loss to the flow taken in was large when the flow was small, and decreased suddenly with the increase of it, becoming constant by its respective value particular to each canal. Thus this critical discharge is the least one which may be conveyed by the canal most economically.

(3) In case of a new trapezoidal canal of 75 cm in upper width, 40 cm in bottom width, and 35 cm in depth, it was advisable to deliver a discharge larger than 28 *l*/sec.

(4) In case of an old rectangular canal of 80 cm in bottom width and with larger discharge than 17-20 *l*/sec, the percentage of the loss became approximately constant.

(5) It was known from the comparison of new and old canals that the loss of an old canal was remarkably less than that of a new one. As the above two canals are different in slope, condition of canals, etc., the author couldn't compare them as they were in this survey, in case of 42 *l*/sec, they were compared by converting to the same distance, the result of which showed that the loss of the new canal was more than 6 times as large as that of the old one.

(6) From the above it was advisable to expedite sedimentation of silt and to contrive to bring the canal into a state of an old one for the maintenance of the canal.

(7) The author is going to clarify the percolation loss by surveying percolation loss from the canals and practising experiments concerning the size of every canal, texture of soils, etc.

98. Statistical consideration on water-warming reservoir. Masuji AKIBA and Shozo NOGUCHI (Rept. Special Committee, Sci. Promot. Soc. Japan 1953)

The authors made a statistical consideration on regional classification, structure, effect of water-warming, etc., on the basis of observed data of 23 water-warming reservoirs, undertaken by the authors from 1949 to 1951 and planning data for 1951 about 61 reservoirs made by the Ministry of Agriculture and Forestry.

These regions where it is necessary to warm irrigation water by water-warming reservoirs in our country are classified into the following three types from the observed data:

(1) Regions where both the atmospheric temperature and temperature of irrigation water taken in are lower than the critical water temperature (about 20°C) necessary for rice-crop.

(2) Regions where water temperature is higher, but the atmospheric one is lower than the critical water temperature.

(3) Regions where the atmospheric temperature drops at night, thought it is pretty high in the day time. The planning data, viewed from statistical standpoint, gives us the following information:

Water areas ranges from 0.084 ha (smallest) to 3.09 ha (largest), the average being 0.747 ha; Storage capacities from 924 m^3 (smallest) to 43,227 m^3 (largest), the average being 13,192 m^3.

Generally speaking, the water area is smaller in the southern districts, and larger in the northern districts, but the storage capacity is large in Nagano Prefecture and small in Hokkaido.

The ratio between irrigated area and water area is 40-80, the average being 58.9.

Even in case of such worst condition as paddy fields are destroyed for the purpose of constructing reservoirs, the increase of production amount due to the above project is much greater than that of the destroyed paddy field, which is quite payable economically.

The construction cost of a water-warming reservoir is ¥1,000,000-¥5,000,000, and the working expense per *tan* ¥3,000-¥10,000, the average being ¥6,000.

As the average cost per *koku* throughout the country is ¥13,800, the working expense can be repayed in only 2 or 3 years, assuming the rice price ¥7,500 per Koku, this fact shows that the above projects are quite payable economically.

99. Some problems on natural drying of clay clods. Fusanobu SHOJI (Journ. Agr. Machin. Soc. Japan **13**, 27-33, 1952)

The author practised some experiments on clay clods by hanging up clay blocks of different diameters in his laboratory, in order to investigate several natures of clods which will present themselves in process of natural drying.

Number of days (T) which are required to reach a certain moisture ratio after drying began will be expressed by the following formula:

In winter: $$T = 0.022\,D^{1.2}(W-w) + 0.0132\,D^{1.6}\left(\frac{20}{w-5.5} - 1\right)$$

In summer: $$T = 0.0156\,D^{1.2}(W-w) + 0.0132\,D^{1.6}\left(\frac{10}{w-5.5} - 0.5\right)$$

where D: diameter of clay block (cm)

W: moisture ratio at the time when drying began (%)

w: each moisture ratio in process of drying (%)

Clay clods reach equilibrium moisture at about 5.5% of moisture ratio.

Viewed from the standpoint of drying velocity, even in case of natural drying, the following three stages of drying velocities were acknowledged: Constant rate of drying, first stage of decreasing rate of drying, and second stage of decreasing rate of drying.

And temperature of air and humidity had a great influence upon the drying velocity, which was especially heavy during the course of the constant rate of drying. Generally speaking, soil clods of small diameter had a larger drying velocity than

those of large diameter even in the period of the constant rate of drying.

Of course, clay clods shrink by drying, and, as for color, they gave the least reflection at 30% moisture ratio, presenting the darkest color.

At first the temperature of clods was nearly equal to that of the humid bulb of a thermometer, but it suddenly began to approach the temperature of dry bulb around the critical moisture ratio (20-15%), and became almost equal to it in the end.

100. On the properties of material of plow-blades and cultivator-steels from the metallic structural point of view. Fusanobu SHOJI (Journ. Agr. Machin. Soc. Japan **14**, 20-23, 1952)

The author measured the contained quantity of carbon and the condition of carbonization concerning 50 samples for the purpose of investigating the properties of material of plow-blades and cultivator-steels made of carbonized and hardened steel, which are used at present, from the metallic structural point of view, and, moreover, for the purpose of knowing if there exists any other subject of consideration there.

According to the result, materials in use now are quite miscellaneous in their properties, and some of the carbonized steel contain carbon as much as 0.3-0.4%, and moreover, annealing is not practised, fibrous structure remaining as it is.

The depth of carbonization is almost less than 0.6 mm, and the concentration of it 0.7-0.8 which shows a favorable condition on the whole. But some of them present extraordinary structure.

Hardness of hardened steels is generally more than 55 degrees by the Rockwell C scale, which is considerably high, but operation of subdivision and tempering is not practised for almost all of them.

Such heat treatment as precipitation of troostite by Ar′ transformation is practised. The core is not ferrite, but sorbite structure occupies the greater part. Widmannstätten structure and hardening crack are seen, too.

101. Anti-foaming method in evaporating can for salt-making. Jiro SUGI and others. (Bull. Soc. Salt Sci., Japan **5**, 1952)

When boiling is practised by adding activator as an anti-foamer, regulation of water level will easily be done; and salting-up, and hours and times for washing decrease, as the result of which heat economy and uniformity of crystal size of table salt will be realized. It was, however, confirmed that the above method had not any bad effect upon the evaporating amount, etc.

102. On mechanism of salt-field, the so-called "Irihama" (Fifth Report). On behavior of ground water in ground of salt-field. Jiro SUGI (Bull. Soc. Salt Sci., Japan **6**, 1952)

The author practised a field experiment concerning how the ground water level of salt-field within the embankment is influenced by ebb and flow of tide, and the following result was obtained:

(1) The fluctuations of the percolating point of sea-water and the depth of stream-line will differ according to the distance from the embankment, and they are approximately constant near the embankment, but the ratio decreases as the distance from the embankment becomes larger.

(2) The lower the ground water level and the larger the hydraulic gradient become, the deeper part the stream line passes through.

(3) As to the relation with the dimension of embankment, it has a relation rather within the position of the bottom level of embankment and the ground level than with the breadth of it.

(4) As to the springs gushing out from the ground surface by the upward tendency of the percolating stream line, the higher the ground water level, the more upward streams become.

(5) The stream lines will become laminar flow, and will not mingle with one another.

(6) Distribution of velocities of stream lines under the embankment tells us that it has a peculiar nature that the stream velocity will not become small suddenly at a certain depth.

(7) The author expressed the interrelation between the percolation water and the saturation length, and that between the depth of the peculiar point mentioned above and the hydraulic gradient by a correlation coefficient.

From these results we know that the water quantity and the wetted area is contrary, which has but little effect, and it is also perceived that the hydraulic gradient and the depth have a close relation between themselves.

103. On clay ground (First Report). On dryness of clay ground and influence of ground water upon it. Jiro SUGI and others. (Bull. Soc. Salt Sci., Japan **6**, 1952)

The author practised an experiment on the mechanism concerning dryness of clay ground, and its influence upon the ground water, and the following results were obtained:

(1) Influence of dryness of the ground reached 3 cm in depth from the ground surface both in one day or 3 weeks experiment, not withstanding the existence or not of the ground water.

(2) The ground water will supplement up to 3 cm from the bottom and percolate in case when it is drying or not, but the quantity supplemented while drying is not going on is very little. Accordingly, in case of clay ground of the flow type, or of salt-fields by sunshine, 6 cm in depth will be sufficient when it is of good quality, but it is better to make the total depth, 8-9 cm by establishing an intermediate layer 2-3 cm.

104. Fundamental experiment on salt extracting apparatus with settling legs. Jiro SUGI and others. (Bull. Soc. Salt Sci., Japan **6**, 1952)

Settling legs are equipped with at the lowest part of the cone of a salt crystalizing can, and the crystallized particles can be made uniform to some extent by sending the liquid having a constant velocity by a pump from the bottom of the legs, and by giving back smaller crystallized particles and impurities to the crystallizing can. Thus the purity of salt will be enhanced by removing impurities, and moreover, it will be produced continousuly by giving back the flowing liquid from the settling legs by pumping.

Thus the relative value of Re-ϕ (Re: Reynolds coefficient; ϕ: resistant coefficient) was experimentally obtained, by which the velocity of the supplied liquid could easily be obtained by d-V_t experiment in the liquid in which η and ρ are

different. (η: velocity coefficient, ρ: density of the liquid), or from the result of calculation. (d: density of a particle; V_t: ultimate settling velocity).

105. On removal of calcium of sea-water by bicarbonate. Jiro SUGI and others. (Bull. Soc. Salt Sci., Japan **7** [2], 1953)

As a method of removing scales the authors added $Mg(OH)_2$ to the brine in case of boiling it, and at the same time made $Mg(HCO_3)_2$ combine with $CaSO_4$ in the sea-water, which would be generated by breathing CO_2 gas into it. Thus $Ca^{\cdot\cdot}$ in the form of $CaCO_3$ was removed by this method devised by the authors. The reaction was speedily and completely performed by adding an accelerator.

The method is summarized as follows:

(1) The following chemical reaction was assumed to occur.

$$Mg(OH)_2 + 2CO_2 = Mg(HCO_3)_2$$
$$Mg(HCO_3)_2 + CaSO_4 = Ca(HCO_3)_2 + MgSO_4$$
$$Ca(HCO_3)_2 = CaCO_3 + H_2O + CO_2$$

(2) The reaction is accelerated by composition of $CaCO_3$. In this case 1 g per *l* of brine will be sufficient.

(3) NaCl will expedite, and $MgCl_2$ and $MgSO_4$ counteract the reaction respectively.

(4) 30°-40°C is the optimum temperature for the reaction.

(5) 10-30% will do as the dilution of CO_2 gas to be used, too.

(6) By taking away the bubbles which will generate upon the liquid in course of the reaction, the reaction will be expedited.

(7) Addition of equivalent of $Mg(OH)_2$ 3 or 4 times as much as that of Ca of sea-water to be disposed will make the disposition finish in 1 or 1.5 hr.

106. Report on concentration of NaCl contained in the River Ibi. Toshio YAHATA (Journ. Agr. Eng. Soc. Japan **20**, 308-313, 1952)

In order to secure the irrigation water as replenishment use, the Miye Irrigation Project has been programmed, as the water source of which the river Ibi is scheduled. The projected site is located at a point about 16 km upstream from the river mouth and lies at the tidal part of the river.

It is feared that the harmful water to the growth of rice-plant may be pumped up at the site in the midst of the irrigation season which falls on the lowest water level of the river.

The author took up the investigation of the river discharge and the amount of NaCl concentration of the first part of September, 1951, in compliance with the request of Miye Prefecture, and the following information was obtained:

(1) The ebb and flow at the site in question is delayed about 2.5 hours, comparing with those at the river mouth.

(2) At about the time of flow mentioned above the maximum concentration of NaCl sometimes reaches up to 0.2%.

Such being the case it is concluded that pumping-up of irrigation water at the projected site is not advisable.

The water bottle of the Kitahara type and an electric apparatus which the author devised were both employed for the above survey.

107. Research on pulling stumps (First Report). Actual state of pulling stumps at the foot of Mt. Iwate. Fujio YAMAZAKI, Toshio YAHATA and others. (Journ. Agr. Eng. Soc. Japan **20**, 1-14, 1953)

Stump pulling is the most laborious work which requires much time among various reclamation operations, and so settlers are apt to postpone the work as late as possible, for they have no sufficient labor. As the result you will find many stumps remained in the farm without being disposed of even to-day when 6-7 years have already elapsed since the settlement started. The stumps have been obstacles to settlers' farming, becoming thus a scourage to expediting their farming management.

In order to solve the stump-pulling problem at the settlements, its actual state should be clarified at first, and then the technical investigation made. From such view-point, the authors practised a survey concerning the present state of pulling stamps by means of enquête and direct hearing to settlers at the foot of Mt. Iwate, the result of which was as follows:

(1) The average allotment area per household was 3.3 ha at the foot of Mt. Iwate, the ratio of reclamation being 76% which is broken down as follows:

Upland fields where stump pulling was completed	43%
Upland fields where stump pulling has partially been undertaken	25%
Upland fields where stump pulling is not started yet	32%

(2) On an average, 140 stumps per *tan* (0.1 ha.) are remained in the upland fields where stump pulling is not started yet.

(3) About 44 persons per *tan* will be required for reclamation of wooded land, and percentages by operation are shown as follows:

Clearing	16%
Burning	1%
Disposal of sundries	14%
Stump pulling	25%
Plowing	23%
Soil crushing	13%
Others	8%

in which stump pulling requires labor most.

(4) Settlers adopt such a simple method of reclamation as to plow and crop their field without pulling out stumps which requires much labor, for they have to widen their cropped areas as quickly as possible in order to receive the government subsidy and to get self-supply food stuffs.

As the result introduction of livestock into their farming becomes difficult, and they are obliged to depend upon human labor which requires labor as much as 3 or 4 times of livestock farming.

(5) The greater part of operation of stump pulling was practised by human power, and only 20% was done by power of livestocks and tractors.

(6) 70% of transportation of stumps pulled out was practised by human power, and 30% livestock. 2/3 of the former was carried away by shoulder, and only 1/3 transported by wagons.

(7) Stump-pulling is very easy, if roots decay after several years. Progress of decay of roots depends upon kinds of trees. Japanese red pine (*Pinus densiflora* S. et Z.), *Hannoki* (*Alnus Jaaponica* S. et Z.) and acacia will decay quickly, and *Kunugi* (*Quercus acutissima* Carr.), Japanese chestnut (*Castenia exenata* S. et Z.),

Japanese larch (*Larix Kaempferi* SARG.) and *Quercus glandulifera* are slow in decay.

(8) In order to prompt decay of roots, settlers will adopt such methods as "Nipping buds", "Cutting roots", "Covering stumps with soil, grass, trash", etc.

(9) Stump-pulling is chiefly practised during the time ranging from the end of the snow-melting period in spring to the beginning of the seed-sawing period (March-April), and from the beginning of the slack season in winter to the period when snow begins to accumulate (November-December).

(10) 80% of stump-pulling was practised by domestic labor, a little over 10% by hired labor, and that by cooperation with associations or neighbors was less than 10%.

(11) Shortage of labor and fund on the part of settlers are chiefly responsible for the delay of stump-pulling operation.

108. Research on pulling stumps (Second Report). Characteristics of root system of various tree kinds viewed from stand point of stump-pulling. Fujio YAMAZAKI, Toshio YAHATA and others. (Journ. Agr. Eng. Soc. Japan **20**, 15-26, 1953)

Though difficulty of stump pulling depends upon tree diameter, progress of decaying, wood quality and other various factors, the root system which are different according to tree kinds has a great relation with it among other things.

In case of the same kinds of trees, spreading of roots depends on gradient of land, texture of soil, soil layer, location of ground water, but it is considered that the major characteristics does not alter. If the characteristic of root system of every tree kind is clarified, logical application of force to every tree in case of stump-pulling will become clear, and, consequently, it will be possible that the method of stump-pulling suitable to a kind of tree may be devised or selected.

From such view point, the authors investigated the condition of root system of 10 kinds of trees, the result of which is shown in the following chart:

Root System / Tree Kinds	Main-roots			Secondary-roots		Numbers of Root
	Tap-root	Subtap-root	Lateral-root	Hangdown-root	Stringlike-root	
Japanese red pine	++	–	++	–	–	+
Japanese cedar (*Cryptomeria japonica* D. Don)	+	0	+	+	–	+
Pea-fruited cypress (*Zhamaecy paris pisifera* S. etz. Z.)	– –	– –	+	+	–	–
Kunugi (*Quercus acutissima* Carr.)	–	–	++	++	++	++
Nara (*Quercus glandulifera*)	–	–	++	+	++	++
Kashiwa (*Quercus dentata* Thumb.	– –	– –	+	0	++	+
Cherry tree	– –	–	+	0	0	–
White birch	– –	– –	+	+	++	++
Japanese larch (*Larix Kaempferi* Sarg.)	0	– –	++	++	0	++
Keyaki (*Zelkowa Serrata* Makino)	– –	0	+	++	+	+

Note: ++ very many, + many, 0 mean, – few, – – very few.

By comparing this chart with the experience of stump-pulling test, some judgement will be given concerning difficulty and suitable method of stump-pulling.

(a) *Nara*, Japanese larch, *Kunugi*, etc. which have many roots are more difficult in pulling out stumps than Cherry-tree, Pea-fruited cypress, etc. which have less roots.

(b) Such shallow-rooted trees as Pea-fruited cypress, *Kashiwa*, etc. are generally easy in pulling out stumps comparing with such deep-rooted one as Japanese cedar.

(c) It is very difficult to pull out a stump by applying horizontal force to such a tree as Japanese red pine, which has a special large tap-root.

In such case it is logical to pull out a stump by revolving tap-root by livestock, after the operation of cutting off the lateral-roots by human labor.

(d) Though Japanese red pine which has comparatively shallow lateral-roots can be disposed simply, *Nara* and Japanese larch which have lateral-roots spreading skew are difficult in doing so. In such case the resistance to pulling out stumps is very large, even if horizontal or revolving force is applied to such stumps.

V. Fisheries

Abstract Nos. 109—137

109. A method to mark the time in the scale and other hard tissues of fishes to see their growth. Yoshio HIYAMA and Ryushi ICHIKAWA (Jap. Journ. Ichthyology **2**, 156-167, 1952)

In order to measure the periods of annual ring formation and the growth rate of fishes, a method to mark the time in the scale and other hard tissues was established by the authors. Its principle is as follows. Lead which is introduced into the blood of fishes by the injection of lead-acetate into their dorsal muscle, deposits on the scale and other hard tissues instead of calcium. After death of them this deposition can be detected to be a blue line by sulpheration and afterwards gilting of gold. This line indicates the growing margin of the calcified tissues at the time of injection. The amount of doses was altered variously and the optimum amount is decided to be 0.01 mgr. per 1 gr. body weight using 0.1% solution. The authors applied this method not only to the gold fishes in aquaria but also to the tagging experiment of Japanese common goby. To see the lead deposition in scale, the transverse section of scale was needed. (But the authors succeeded to observe the clear lead line in scale from the surface view without any section. This is reported in another paper.) Fin rays, operculer bones and vertebrae were also examined to see the growth by this method. But, in case of otolith, this method could not be applied as the lead deposition did not occurred in this tissue which consisted of calcium carbonate crystals. The influence of this injection to the rate of recovery at the tagging experiment did not exist seeing the recovery of injected and non-injected fishes. The exact growth rate obtained from this experiment is reported in next paper of the authors.

110. Individual differences and degree of difficuty in age determination of six fish species. Yoshio HIYAMA, Takaya KUSAKA and Yukio NOSE (Records of Oceanographic Works in Japan **1**, 138-148, 1953)

The authors wanted to know about the extent of differences of the results of

age determination made by different persons, and also about the differences of it by species of fish. In June of 1952, a class of nineteen students taught by the authors were mobilized as the age readers to supply the data for us pretending as it is an examination, after several weeks training on the technique and lectures on this matter. The materials were:—(I) 44 scales of the herring, *Clupea pallasi*; (II) 42 scales of the saury, *Cololabis saira*; (III) 41 scales of the common goby, *Acanthogobius flavimanus*; (IV) 43 otoliths of the Japanese sole, *Microstomus kitaharae*; (V) 50 scales of the yellow fin tuna, *Neothunnus macropterus*; (VI) 47 scales of the crucian carp, *Carassius carassius*. These 267 samples were prepared for microscopic observations, being each sample set between two slide glasses without any staining.

At the first step, the result of the age determination by individual person is arranged to indicate a sort of age composition of the certain fish population. These nineteen curves of age composition, each of which is obtained by individual observer, are expressed together in a graph. The herring, by the feature that all these nineteen curves run close together, is supposed to be the species, age determination of which is most easy in all six.

Moreover, by the mathematical method, the degree of difficulty of age reading regarding particular fish species was obtained about these six species. And the result way very similar to the impression by the graphic expression

The frequency of coincidence of age value of the same sample obtained by these various observers were quite rare. Only one examples among 267 was found that all nineteen persons reported the same age value, and it happens in one of herring scale. In an extreme case of the crucian carp scale, only four persons read the coincidental figure age.

Besides, the personal tendency in age reading is expressed based on the deviation from the average mathematically.

This study cleared out ho wthe age determination which had been obtained as a basic factor for fisheries biology, had contained the personal errors.

111. A consideration on the causes of change of locomotion activity of fish by aquarium experiment. Takaya KUSAKA (A Special Publication of the Japan Sea Regional Fisheries Research Laboratory on the 3rd Anniversary of its Founding, 165-173, 1952)

The experiment was undertaken to see the change of locomotion activity of fish at various time of day, and its change caused by feeding and other conditions.

Aplocheilus latipes (Japanese rice paddy minnow), 10 individuals of 2.5 cm. long in average, were liberated in the glass tank (90 cm. long, 36 cm. broad and 25 cm. deep). The observer counted the number of fish in every minute—he called the frequency of transfer—which pass across the imaginal surfaces which divide the tank into four similar quarters both horizontally and vertically.

Consequently, the locomotion activity of the minnow, both horizontal and vertical, enumerated by average frequency of transfer has effect by time of day of its observation. Vertical transfer is more frequent in morning and getting less toward evening. Horizontal transfer is most frequent in noon and less in morning and evening. The effect of feeding is the remarkable increase of locomotion activity on horizontal transfer and it continues about one hour after feeding, and it is not obvious

on vertical. The less obvious effect is recognized by the difference of weather condition. The water temperature where fish kept during the experiment might be one of the dominant factors to effect it. However, the range of water temperature in these experiments was narrow (21-28°C) to see it enough to be significant.

The author recognized the above evidence by consideration of the result of a series of experiment. However, this should be true only about the fish having the same character as *Aplocheilus,* and it is reasonable to suppose that the result would be different if the same sort of experiments are done about the nocternal fish or other peculiar habit.

The author can say at the present step of the study, merely that the fact that the change of time of day has such a manifest effect to the fish activity, is originally caused either by the change of lightness of the place, experiment caused by change of time of day, or by some other unconceivable factors having diurnal periodicity, without doing such experiments in a dark or constantly light room.

112. Some experiments on the shoaling reaction in *Oryzias latipes* (TEMMINCK et SCHLEGEL). Yoshio NAKAMURA (Bull. Jap. Soc. Sci. Fish. **18**, 93-101, 1952)

In order to make clear some points concerning the studies on animal aggregations which have advanced since W. C. ALLEE, I used about 200 himedakas as experimental animal in a special apparatus. The himedaka is a red variety of medaka which is the Japanese common name for *Oryzias latipes* (TEMMINCK et SCHLEGEL).

The apparatus is made exclusively for the purpose of knowing the shoaling reaction of the fish. Namely two cylindrical transparent glass pails are placed concentrically, the smaller one inside the larger one, in a tin basin. Between the side walls of both pails two black rectangular rubber plates are inserted respectively at both sides on the pencilled bottom diameter. The water is put into both pails so as to be at the same level inside and outside the smaller pail. Fishes can swim freely in the smaller pail, while they cannot pass from one side to the other of the ring-shaped area between the two pails because the area is separated into two parts by the two rubber plates.

Fishes in the smaller pail and in the ring-shaped area are called "reacting individuals" and "stimulating individuals" respectively, because the former are reacted upon by the latter and the aim of this experiment is to know how this happens. The number of the reacting individual is 1 at any time, while that of the stimulating individual ranges from 1 to 128.

For the purpose of judging whether there is any stochastical difference or not between or among the figures in the results obtained, that is, whether the stochastical null-hypothesis that they are the samples extracted at random out of the mother group is to be abandoned or not under 1% level of significance, Motosaburo MASUYAMA's up-to-date techniques in stochastics were adopted, the standpoint being taken that those figures do not make a normal distribution, but make a binominal distribution.

Through the experiments the following results were obtained. But there would be a lot of room for further analysis and argument on these points.

(1) WEBER-FECHNER's Law can be induced from the correlation between the reacting rate and the number of stimulating individuals.

(2) The reacting rate is controlled chiefly by the number of stimulating in-

dividuals, but in a range of the number it is dominated by the momentum of the stimulating individuals as well as by other factors, while it remains almost unchanged so far as it is beyond a certain limit, no matter how much the number of stimulating individuals may be increased.

113. On the sexual maturity of the sperm whale (*Physeter catodon*) found in the adjacent waters of Japan. II. Masaharu NISHIWAKI and Takashi HIBIYA (Sci. Rep. Whales Research Inst. **5**, 121-124, 1952)

The material which serves as the basis for this study covers all the sperm whales (fifty-five males and one female) caught in the waters east of the Bonin Is. during the period March 17-June 10, 1951.

Judging from histological examination and others, the male sperm whales in the adjacent waters of Japan reach sexual maturity at body length less than 38 Eng. ft., confirming the theory proposed in the previous study.

The only female sperm whale caught during the expedition measured 38 Eng. ft. in length. Her twelve corpora lutea were all old.

114. On the age and the growth of teeth in a dolphine, (*Prodelphinus caeruleo-albus*). I. Masaharu NISHIWAKI and Teiji YAGI (Sci. Rep. Whales Research Institute **8**, 133-146, 1953)

Age data are essential to the analysis of whale stocks, but a perfect method has not yet been developed.

We attempted to discover any relationship that may exist between the stripes in the section of the dentine and the age of the toothed whales in the hope that a method of age determination for these whales may be derived from the understanding of such relationship, so as to facilitate the analysis and conservation of their stocks.

In the present study, a species of dolphin (*Prodelphinus caeruleo-albus*) was used for a preliminary purpose.

(1) With the view of determining the rate of formation of stripes in the dentine of the dolphin, teeth of four dolphins were stained *intra vitam* by injectioning lead acetate into the dorsal muscle.

The dose was 5 mg of lead acetate per kg of body weight. But nothing more than preliminary informations were obtained, for successful observations were made only on few individuals. This was chiefly due to the fact that the dolphins did not survive, after the injection, long enough for their teeth to growth to any considerable extent.

(2) By studying the teeth of prenatal and postnatal individuals, it was made clear that this species is monophiodont. Its dentition is bilaterally symmetrical, and hardly differs in the upper and lower jaws. The dental series consists of homodonts of very similar sizes. Except the very small teeth near the anterior end of the dental series, all teeth of an individual show the same striped pattern in the section of their dentine. And so, one may reasonably designate in every individual a tooth in a fixed position of the dental series as the representative of all teeth of that individual, and compare only these teeth with one another, when the teeth of different individuals are to be compared.

(3) In the longitudinal section of the teeth, the dentine is seen to be divided in two parts by a line unstainable with haematoxylin: the striped pattern is seen

in that part formed after birth, and not in the dentine formed during the foetal period. It was deduced that this line is the neonatal line, which is produced by the change in the nutritional conditions concurring with the transition from the foetal life to the suckling period.

(4) General biological observation were made on 90 adults and 15 foetuses. As far as available data indicate, this species is born at body lengths between 100 cm and 120 cm, and reaches sexual maturity at the body length around 215 cm. But the data are so scarce that this must be considered as a preliminary conclusion.

115. On the rearings and metamorphoses of four important barnacles in Japan. Reijiro HIRANO (Journ. Oceanogr. Soc. Japan **8**, 139-143, 1953)

Although considerable attention has been paid to the development of barnacles, we have as yet very little data, and it has been impossible to discriminate each nauplius stage or to identify the barnacles in their larval stages.

In an attempt to secure some knowledge enough for us to do the discrimination, the artificial rearings of four important species in Japan were carried out in our laboratory and observation has been made on their metamorphoses. These species were as follows ; *Balanus amphitrite communis*, *Balanus trigonus*, *Balanus tintinnabulum rosa* and *Tetraclita squamosa*.

The rearings were carried out in a thermostat under the temperature most suitable for each species attachment. They were fed with the artificially cultured marine planktonic diatom *Skeletonema costatum*.

All of these four species of barnacles, without exception, passed through seven nauplius stages in their development from hatching to the cypris stage. The time found necessary to complete these developments ranged from 5 to 10 days.

During the development the increase of number of spines of the first appendage occurred regularly as shown in the table, so the number of spines can be the criterion in discriminating each nauplius stage. There are 13 spines on the first appendages in both 6th and 7th stages, but the latter (metanauplius) can be discriminated from the former by the appearance of a pair of compound eyes on both sides of a nauplius eye.

On the other hand, the basipodites of the second appendages have undergone no changes during the development. By this fact, the structural differences in the basipodites of second appendages can be used as the key for classifying the species of nauplii. The structure of the caudal horn can be used also for the same purpose.

Table. The number of spines on the first appendages and on expodites of the second and third appendages.

Stage	1st appdg.	Ex. 2nd appdg.	Ex. 3rd appdg.
I	8	5	4
II	8	7	5
III	9	7	5
IV	10	9	5
V	12	11	6
VI	13	12	6
VII	13	12	6

116. Studies on sedentary marine organisms. I. Seasonal variations in the attachment and growth rates of barnacle cyprids in Aburatsubo Bay, near Misaki. Reijiro HIRANO and Jun OKUSHI (Bull. Jap. Soc. Sci. Fish. **18**, 639-644, 1952)

The daily attachment and seasonal growth rates of barnacle cyprids, as they are factors governing the development of barnacle population, have been investigated in Aburatsubo Bay. The results obtained are as follows:

(1) The number of the cyprids attaching daily varied from 0 to 59 per 900 sq. cm. and each species showed a heavy cyprids attachment in different temperatures of sea water. That is,

Balanus trigonus	from 20°C to 25°C
Balanus amphitrite hawaiiensis	from 24°C to 27°C
Balanus amphitrite albicostatus	from 21°C to 24°C

(2) No relation between the frequent peaks in cyprids settling and change in salinity was established.

(3) The ratio of barnacles found on the monthly collectors to cyprids attached in each monthly period may be explained that the bacterial slime film, which is formed on glass surface submerged in the sea, provides an environment more suitable for attachment than a clear surface.

(4) Rate of growth was studied only about one species, *Balanus amphitrite hawaiiensis*, and its seasonal variation in the growth rates of length and breadth was observed, but barnacles, settled in each seasons, sexually matured in about 40 days similarly in spite of their different sizes.

117. On culture of *Venerupis semidecussata* (REEVE) by NaCl solution. Yoshiyuki MATSUE and Yasuo ITAZAWA (Collecting and Breeding **14**, 234-238, 1952)

Asari (*Venerupis semidecussata*), one of the most popular edible clams in Japan, is known easily to be kept alive for a while in the water dissolved NaCl alone. The culture method is commonly used at our home kitchens before cooking of the clams with the purpose of making them ejected mud and sand containing in shell cavities.

Though the cleaning method is usually successful, not a few cases are also observed in which the treatment ends entirely in failure, viz. every clam keeps its shell tightly closed in the solution and don't show any active movement. Either inferior vitality of the animal or unsuitable concentration of the medium, or moreover, both together are generally considered to be the cause or the causes of such failure, but without any definite authorities.

Investigations upon the culture method were carried out by the authors and were able to obtain some notable results on the point, the summaries of which are as follows:

By the NaCl solution culture the natural sea water contained in the shell cavity of the clam has an important meaning. The clam can not reveal any active behavior in the pure NaCl solution. For the activation of the animal some little amount of sea water is necessary to be mixed with the culture medium and the indispensable sea water must be supplied from the clam itself. The minimum necessary value of the mixing ratio of sea water against surrounding medium is proved to be about 2%, when animals are in normal vitality but less robust ones need more sea water. Naturally, the more sea water to be mixed with the better for the animal.

It is concluded from those results that success or unsuccess of the culture method mainly depends upon both the amount of sea water contained in shell cavity and the

amount of aquarium water added to. The quantity of sea water contained in a clam seems to be variable by many factors and differs actually in each case. Therefore, the key point of the culture method is reduced to only one point that we prepare possibly little amount of NaCl solution as aquarium water. Needless to say, there is no problem when the addition of sufficient amount of other sea water is possible.

As to the suitable concentration of NaCl it is proved that the value of 25 g/l to be most favorable, though the clam can stand for a comparatively broad range of the concentration.

118. Some substances in sea water which disturb the colorimetric determination of phosphate. Aritsune SAEKI (Journ. Oceanogr. Soc. Japan **8**, 73-78, 1952)

It is known that Cl ion in sea water diminishes the phosphomolybdenum blue on the phosphate determination by ATKINS' method, so the correction must be made on the value obtained. These correction factors are varied by the authors from 1.00 to 1.35. This variation shows that there might be some other substances which disturb the colorimetry. It has already been pointed out by some investigators that these substances are colloïdal iron, copper and colloidal parts of mud. Some experiments were made on this problem, and it was found that nitrite in the water when contained more than 50 γ/l in sea water or 30 γ/l in land water, too, diminishes the phosphomolybdenum blue and disturbs the determination. So it is desirable that stannous chloride solution is dropped in fifteen minutes after acid-molybdate reagent is added, when the water contains nitrite more than above mentioned values.

It is often observed that the phosphate content of the water added with preservatives increases in the time of storage since bottled to analysis. Phosphomonoesterase, which liberates ortho-phosphate from organic phosphorous compounds, is detected in the sea water and plankton tow water. The action of that enzymes in the water containing plankton or other organisms, is suggested one of the reasons of this phenomenon.

119. On the life-history of *Kjellmania Arasakii* YAMADA and its systematic position. Seibin ARASAKI and Koji NOZAWA (Bull. Jap. Soc. Phycology **1**, 5-9, 1953)

Kjellmania Arasakii YAM. sp. n. (in Japanese, Samezu-gusa) is a rare brown alga in Japan, and it is found restrictedly on the coastal shore near Tokyo-City during the limited seasons of the winter-time. The alga is slender, branched and filamentous, and produces zoospores in pluri-locular sporangia. The zoospore germinates directly, and grows into a protonema-like microphyte through a *Laminaria*-type process. After a while of dormance, the protonema protrudes many up-right minor threads, on these a large many plurilocular sporangia are made in a form of dense clusters. The zooids liberated from these sporangia are gametes, and conjugate pairly. The zygote develops into a filament of *Kjellmania* plant which is, at first, uni-seriate, and afterwards becomes multi-seriate by intercalary longitudinal divisions. From these, it is evident that in the life-cycle of *Kjellmania*, the macro sporophyte alternates with the micro gametophyte, and the sexual reproduction is gametogamous. From the view point of the morphological structure, it is suggested that this alga may phylogenetically be higher than *Dictyosiphon*.

120. On the *Eisenia* found in Japan. Hiroshi KUNIEDA and Seibin ARASAKI (Proc. Seventh Pacific Science Congress **5**, 1953)

The Japanese *Eisenia* (in Japanese, Arame) has been said to belong to one and the same species, *E. bicyclis* (KJELLM.) SETCHELL. But detailed investigations investigations indicates that the Japanese species may be separated into, at least, two different groups by following morphological characters.

	A group	B group
External feature of the ligule	broader and shorter, 110–460 mm (M. 282) in L. 23–88 mm (M. 48) in Br.	narrower and slender, 37–1,020mm (M. 488) in L. 7–110mm (M. 44) in Br.
Surface view of the ligule	reticulately undula to-rugose, but the rugae are not so distinct	logitudinally rather than reticulately rugose, and the rugae are distinct
Margin of ligule	bears numerous, well-developed leaves	bears serrate protuberances, but no well-developed leaves
Number of ligules on each forked branch	7–39 (M. 18)	4–65 (M. 20)
Zoospore	wants a stigma	has a definite stigma
Distribution	mainly in the northeastern parts from Suruga Bay	mainly in the southwestern parts from Suruga Bay

From these differences, we wish to call A-group *Eisenia bicyclis* SETCH. (in Japanese, Arame) and B-group *Eisenia arborea* ARESCH. (in Japanese, Sagarame). From this, we may safely state that *E. arborea* is not only limited to California, but its area of distribution includes the southern part of the Japanese coast.

121. Animal protein factor (APF) and vitamin B_{12} in marine products. III. Seaweeds (Part 2) and others. Yoshiro HASHIMOTO and Tokuji SATO (Bull. Jap. Soc. Sci. Fish. **19**, 45, 1953)

The vitamin B_{12} content of about thirty species seaweeds determined by *Euglena* method, varied from 0.1 to 4 $\gamma\%$ on fresh basis and about twenty species among them contained less than 1.0 $\gamma\%$. The thin or finely branched seaweeds contained generally much vitamin B_{12}. Asakusanori (the air-dried product of *Porphyra tenera*) was very rich and contained from 13.2 to 18.6 $\gamma\%$ B_{12}. The utilization of marine algae as APF feed for domestic animals may be confined to only few species, if possible.

Several zoo- and phytoplankton were also determined on their B_{12} content; *Dephnia pulex* 3.8, *Mysis sp.* 0.9, *Skeletonema costatum* 8.5, *Microcystis sp.* 4.8, *Scenedesmus obliquus* 0.15 and *Chlorella elipsoidea* 2.5–3.1 on fresh basis. Another eight preparates of air-dried *Chlorella* contained from 4.2 to 8.9 $\gamma\%$ B_{12}. The relatively high content of a diatom and some fresh-water unicellular algae, such as *Microcystis* and *Chlorella* is noticeable.

The bottom mud of fresh-water pond was very rich in vitamin B_{12}, perhaps produced by soil micro-organisms. (0.8-3.1 $\gamma\%$ on fresh basis).

122. Animal protein factor (APF) and vitamin B_{12} in marine products. IV Variations in the vitamin B_{12} content of marine animals in the spoilage. Takajiro MORI, Yoshiro HASHIMOTO and Yasuhiko MAEDA (Bull. Jap. Soc. Sci. Fish. **19**, 45, 1953)

The vitamin B_{12} content was determined by *Euglena* method on the digested samples with trypsin. In the spoilage of marine animals, the marked fluctuations of vitamin B_{12} content were observed. As the recovery test of vitamin B_{12} with the putrid fish meat gave the same result as in the case of fresh meat, the variation must be considered as the increase or decrease of vitamin B_{12} itself. As a whole, B_{12} content decreased generally when the round fishes were allowed to spoil; for instance, the B_{12} content itself. As a whole, B_{12} content decreased generally when the round fishes were allowed to spoil; for instance, the B_{12} content of carp liver decreased from 8.0 to 0.9 $\gamma\%$ in 36 hrs. and the dark-colored meat of jack mackerel from 11.2 to 0 $\gamma\%$ in 3 days in the most marked cases. The decrease was less or, on the contrary, the considerable increase was observed in some cases, when fishes were spoiled in the state of minced flesh or organs. The minced clam meat showed the most remarkable increase, namely, the B_{12} content became four to sixfold in four to nine days putrifaction.

As the variation was prevented by the addition of toluene or by the cold storage at $-2\sim-5°C$, the bacterial synthesis or decomposition seems to be the main cause of it. The vitamin B_{12} splitting enzyme was not detected on the glycerine and water extracts of mackere lliver and carp meat.

123. Comparative studies on the stomachal plates and crystalline style. II. Structure of the stomachal plates of *Dolabella scapula.* Takashi HIBIYA, Eiji IWAI and Yoshiro HASHIMOTO (Bull. Jap. Soc. Sci. Fish. **19**, 1-4, 1953)

The stomachal plates are found in the gizzard of many opisthobranchs. In previous paper, we reported the presence of digestive enzymes in the plates of *Dolabella* and pointed out the important roles played by the plates. This paper concerns with the histological observation on the plates and gizzard of *Dolabella*.

The plate is generally dark amber-colored, composed of very fine and long pillars and has a thick irridescent column in its axial part. The microscopic observation of the plates and gizzard gave a powerful evidence to show the formation of the former from the latter. The digestive enzymes are supposed to be incorporated into the plates in the process of its formation from the gizzard. Eight figures are listed.

124. Comparative studies on the stomachal plates and crystalline style. III. Chemical constituents of the stomachal plates of *Dolabella scapula.* Yoshiro HASHIMOTO and Takashi HIBIYA (Bull. Jap. Soc. Sci. Fish. **19**, 5-7, 1953)

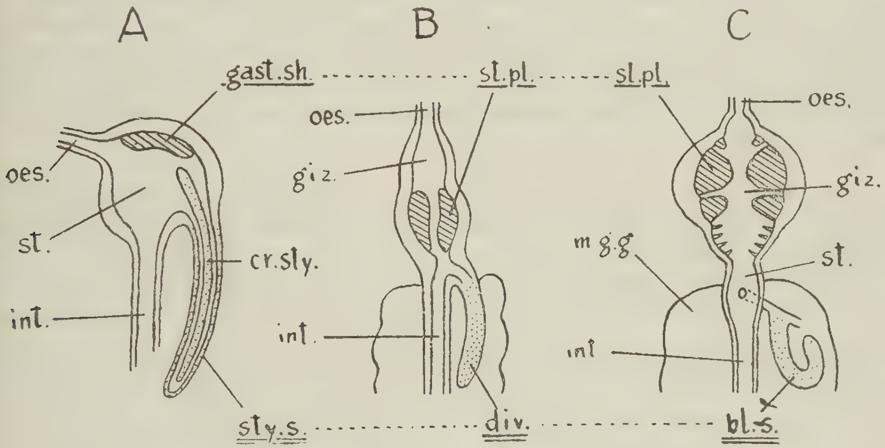
The structural component of the stomachal plates of *Dolabella* was confirmed to be chitin and differs apparently from the crystalline style. The chitin obtained showed the analytical results, 0.14% of ash and 6.17% of nitrogen and produced glucosamine hydrochloride on hydrolysis. The analysis of the plates gave the following results; moisture 80.2, crude fat 0.03, total nitrogen 1.8, chitin 7.3, crude ash 1.6, $CaCO_3$ 0.3 and others 11.0 per cent respectively.

The plates are assumed to be *homologous* to the gastric shield in lamellibranchs in the origin and chemical constituents, whereas they are *homogeneous* to the crystalline style in the function.

125. Comparative studies on the stomachal plates and crystalline style. IV. On the crystalline style of *Dolabella scapula*. Yoshiro HASHIMOTO, Takashi HIBIYA and Eiji IWAI (Bull. Jap. Soc. Sci. Fish. **19**, 67-70, 1953)

The authors discovered the presence of a crystalline style in an opisthobranch *Dolabella*, for the first time. The blind-sac, whose origin and function remained unknown, whereas its presence has been long known, was confirmed to be nothing but the style sac and contain a crystalline style in it. The structure of the blind sac was described in detail.

The relations among the accessory organs of mollusc stomach, such as crystalline style, style sac, blind sac, gastric shield and stomachal plates, were discussed and illustrated in the diagrammatic figures shown in below.



Diagrammatic representation of the relations among the digestive organs in the different species of molluscs.

A. *Donax sp.*, B. *Creseis acicula*, C. *Dolabella scapula*

bl. s. blind sac; cr. sty. crystalline style; div. diverticulum; gast. sh. gastric shield; giz. gizzard; int. intestine; m.g.g. mid-gut gland; oes. oesophagus; st. stomach; st. pl. stomachal plate; sty. s. style sac.

126. Studies on the antioxidants for margarine enriched with vitamin A. II. Takajiro MORI, Kōji NAITO, Yasuo OZAWA and Hirosi KENBO (Journ. Jap. Soc. Food and Nutrition **5**, 180-182, 1953)

The milk components, which were added to margarine to improve the taste and the state of emulsion, were examined on their effects to protect vitamin A in the margarine from oxidation. Their effects were also compared with other synthetic antioxidants.

Cream, skim milk and fermented skim milk were very effective, butter milk next, but butter fat was inactive. The milk components were more effective than NDGA (nordihydroguaiaretic acid), propyl-gallate and α-tocopherol.

However, as the margarine combined with milk components is apt to mold easily, the addition of a fungicide may be necessary.

127. Synthesis of vitamin A and its related compounds. I. Takajiro MORI and Kazuo SHIRAI (Vitamins **5**, 562-565, 1952)

β-Ionylideneformaldehyde (I) was prepared by the condensation of β-ionone with ethyl chloroacetate using alcohol-free sodium methoxide, followed by hydrolysis and decarboxylation with methanolic sodium hydroxide.

The side chain, on the other hand, was prepared as follows; methyl vinyl ketone, obtained by dehydration of β-hydroxyethyl methyl ketone, a condensation product of acetone with para-formaldehyde, was treated with lithium acetylide in liquid ammonia. 3-Oxy-3-methyl-penten-(1)-in-(4), thus obtained, was brominated and then converted into 1-ethoxy-3-methyl-penten-(2)-in-(4) (II) with sodium ethoxide.

(I) was condensed with (II) by means of GRIGNARD's reaction and the product 1-ethoxy-3, 7-dimethyl-6-oxy-9-[2', 6', 6'-trimethyl-cyclohexen-(1')-yl]-nonadien-(2, 7)-in-(4) was partially hydrogenated with palladium in the presence of small amounts of quinoline in methanol. The hydrogenated compound was dehydrated by iodine in petroleum ether and purified by chromatography on activated alumina. The purified substance gave CARR-PRICE reaction and showed absorption maximum at 329 mμ, proving that vitamin A ether was synthesized.

128. Synthesis of vitamin A and its related compounds. II. On the biological activity of synthesized vitamin A ethyl ether. Takajiro MORI and Shōji KŌNOSU (Vitamins **5**, 566-568, 1952)

The synthesized vitamin A ethyl ether, reported in previous paper, was confirmed to be biologically active for rats according to the standard method of U.S.P.

129. Studies on margarine enriched with vitamin A. II. Effects of milk components and some emulsifiers on vitamin A oxidation in margarine. Koji NAITO and Takajiro MORI (Journ. Agr. Chem. Soc. Japan **27**, 265-268, 1953)

As previously reported, the skim milk was very effective in preventing the deterioration of vitamin A added to margarine. In this paper, the main components of skim milk were examined on their protecting powers. Proteins, such as casein, albumin and globulin were proved to be very effective, whereas lactose and the ash of milk promoted the oxidation of vitamin A conversely.

From the above mentioned results, several water-soluble proteins of another origins, such as casein, albumin, globulin, gelatin and gluten were examined and all of them were confirmed to be effective. As these proteins improved the emulsion of margarine, it was supposed that the improved emulsion state would be served to the protection of vitamin A from oxidation. However, monoglyceride, a fat-soluble emulsifier, showed a little protecting power, but when it was used in higher concentration, the vitamin A oxidation increased inversely. Therefore, it is not obvious whether the state of emulsion of margarine has anything to do with the preservation and deterioration of vitamin A or not.

The mechanism of protection of vitamin A by these proteins is now under investigation.

Gelatin was more effective than the ordinary emulsifiers, monoglyceride, lecithin and combination of them.

130. Studies on margarine enriched with vitamin A. III. Effects of water-soluble colloidal substances on vitamin A in margarine. Kōji NAITO and Takajiro

MORI (Journ. Agr. Chem. Soc. Japan 27, 268-272, 1953)

Water-soluble colloidal substances, such as gelatin, casein, sodium alginate, agar-agar, starch, gum arabic and tragacanth, were examined on their effects on the preservation of vitamin A added to margarine and found to be effective except starch and tragacanth. Generally, the water-soluble proteins were more effective than the colloidal carbohydrates.

Both 0.5 or 0.1% of sodium caseinate and combination of 0.5% gelatin and 0.01 or 0.005% propyl gallate were more effective than 0.02% propyl gallate in margarine.

131. Studies on the whale oil. IV. Properties of oils contained in various parts of a whale body. Part 4. On the pacific beaked whale (*Berardius*). Masamichi SAIKI and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. 18, 269-272, 1952)

We studied on the chemical and physical properties of oils contained in various parts of a pacific beaked whale (*Berardius bairdii*). The properties of neutral fats are summarized in table 1. The properties of the unsaponifiable matter and the mixed fatty acids were also reported in the original paper.

(1) Unsaponifiable matter contents of oils from various parts of a body showed remarkable differences each other.

(2) Average molecular weight of mixed fatty acids from head and jaw oil was very small, as in the case of sperm head cavity oil which we reported previously. Stomach content, liver and viscera oils were a little larger than those for bone and blubber oils in the average molecular weight, as observed also in the case of sperm and fin whales.

(3) Iodine value of liver and stomach content oils was very high, whereas head and jaw oil was extremely small.

(4) Acid value of stomach content oil was very low.

Table 1. Properties of neutral fats contained in various parts of a pacific beaked whale body.

Fat from	N_D^{40}	d_4^{30}	Acid value	Sapon. value	Iodine value	Unsapon. matter %
Head and jaw	1.4459	0.9007	0.1	218.0	24.7	18.33
Head and jaw*.........	1.4477	—	0.1	217.2	34.0	17.81
Blubber	1.4560	0.8748	0.3	123.2	87.4	40.05
Bone	1.4532	—	0.3	115.2	75.3	47.09
Viscera	1.4557	0.8799	0.8	132.1	83.7	32.21
Liver	—	—	82.6	182.0	—	18.28
Stomach contents* ...	1.4581	—	4.3	113.5	97.6	42.59

* ......other whale.

132. Studies on the whale oil. V. The composition of fatty acids of the sperm whale oil. Part 1. On the head cavity oil. Masamichi SAIKI and Takajiro MORI (Journ. Agr. Chem. Soc. Japan 27, 188-190, 1953)

Previously, we reported about the chemical and physical properties of oils contained in various parts of a sperm whale body, and found noticeable differences among these oils.

This time, we studied on the quantitative composition of fatty acids of sperm whale head cavity oil. The results obtained are shown in table 1. The following facts were noticeable. The amounts of saturated and unsaturated acids were nearly equal. Very large quantity of lower fatty acids (less than C_{14}) was present.

Table 1. Acids composition of sperm whale head cavity oil.

		Solid fatty acids*	Liquid fatty acids*	Composition	
Saturated fatty acids......	C_{10}	0.67	6.01	**6.68**	48.85
	C_{12}	6.62	16.04	**22.66**	
	C_{14}	9.54	5.77	**15.31**	
	C_{16}	2.74	1.20	**3.94**	
	C_{18}	0.26	—	**0.26**	
Unsaturated fatty acids...	C_{12}	0.42	13.37	**13.79** (−2H)	51.15
	C_{14}	0.80	14.05	**14.85** (−2H)	
	C_{16}	0.69	12.33	**13.02** (−2H)	
	C_{18}	0.26	6.86	7.12 (−2H)	
	C_{20}	—	2.37	2.37 (−2H)	
		22.00	78.00	**100.00**	

* ......by lead salt-alc. method.

133. Studies on the whale oil. VI. The composition of fatty acids of the sperm whale oil. Part 2. On the bone oil and the large intestine oil. Masamichi SAIKI and Takajiro MORI (Journ. Agr. Chem. Soc. Japan **27**, 190-193, 1953)

Table 1. Composition of fatty acids of the sperm whale oil.

		Head cavity oil	Head oil*	Bone oil	Blubber oil*	Large intestine oil
Saturated fatty acids	C_{10}	6.7	3.5	0.6	—	—
	C_{12}	22.7	16.0	2.5	1.0	1.4
	C_{14}	15.3	14.0	6.7	5.0	5.7
	C_{16}	3.9	8.0	9.6	6.5	12.0
	C_{18}	0.3	2.0	2.4	—	1.4
		48.9	**43.5**	**21.8**	**12.5**	**20.5**
Unsaturated fatty acids	C_{12}	13.8 (−2H)	4.0 (−2H)	0.8 (−2H)	—	0.2 (−2H)
	C_{14}	14.8 (−2H)	14.0 (−2H)	3.8 (−2H)	4.0 (−2H)	1.5 (−2H)
	C_{16}	13.0 (−2H)	15.0 (−2H)	23.4 (−2H)	26.5 (−2H)	12.2 (−2H)
	C_{18}	7.1 (−2H)	17.0 (−2H)	26.3 (−2H)	37.0 (−2H)	33.5 (−2.2H)
	C_{20}	2.4 (−2H)	6.5 (−2H)	19.5 (−2.2H)	19.0 (−2.5H)	26.6 (−2.7H)
	C_{22}	—	—	4.4	1.0 (−4H)	5.5 (>2.7H)
		51.1	**56.5**	**78.2**	**87.5**	**79.5**

* ......by HILDITCH and LOVERN.

The composition of fatty acids of the sperm whale bone oil and the large intestine oil has been examined. Considerable differences in the composition of fatty acids are found in the fats from different parts of one animal, indicating a highly selective formation of the fat deposits.

134. Studies on the whale oil. VII. Fatty acids composition of the pacific beaked (*Berardius*) whale oil. Part 1. On the head and jaw oil. Masamichi SAIKI (Bull. Jap. Soc. Sci. Fish. 18, 497-501, 1953)

Pacific beaked whale (*Berardius bairdii*) head and jaw oil has very characteristic properties; i.e. liquid even in the winter, shows very low iodine value and extremely small average molecular weight of the mixed fatty acids. However, no report has ever been published on the composition of fatty acids of this kind of oil. The composition of fatty acids obtained are shown in table 1. The mixed fatty acids consisted of large quantities of saturated fatty acids (68.84%) which were constituted chiefly of lauric and capric acids, small quantities of myristic and palmitic acids and a slight quantity of stearic acid. Almost all of the unsaturated fatty acids were constituted of monoethenoid acids of C_{18}, C_{16}, C_{20}, C_{12}, C_{14}.

The presence of the extremely large quantities of C_{10} and C_{12} acids was very interesting.

Table 1. Composition of fatty acids of pacific beaked whale head and jaw oil.

	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}
Saturated fatty acids............	21.21	31.94	8.15	6.43	1.11	—	—
Unsaturated fatty acids	—	4.01 (−2H)	3.43 (−2H)	7.57 (−2H)	10.34 (−2H)	5.00 (−2H)	0.81

Acids of C atoms less than C_{10} also exist, but trace.

135. Chemical studies on seaweeds. I. Chemical constituents of "Asakusanori" of various sources. Takeshige YAMAKAWA (Bull. Jap. Soc. Sci. Fish 18, 478-482, 1953)

Asakusa nori (an air-dried food product of *Porphyra tenera*) of various sources was analysed on the total nitrogen, crude fat, crude ash and the following pigments, phycoerythrin, phycocyan, chlorophyll and carotene.

The content of these constituents varied most widely by season. Pigment contents reached to maximum in January and the excellent product in colour tone showed high pigment content. The total nitrogen varied in the same way with pigments and seemed to be high in the excellent product in taste.

In chemical constituents, there was no remarkable difference among the products, which were seeded at the different districts and reared at the same station. Similarly, the influence of the rearing district was not so obvious as the case of season, when the laver was cultured at the different districts after seeding at the same ground.

136. Studies on the molecular distillation of whale liver oils. I. Takeshige YAMAKAWA and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. 18, 645-650, 1953)

The preparation of vitamin A concentrate by molecular distillation was tested on two sperm whale and four fin whale liver oils in a laboratory scale. The yield of distillate and its vitamin A potency were tabulated in six tables and the absorption

curves of each fraction were given in nine figures. The potency of vitamin A was corrected according to OSER's method.

(1) The total amount of recovered vitamin A in distillate and residue was higher than that of original oils except one case, owing to the presence of kitol. The largest increase was observed in a sperm whale liver oil and the potency became 141.9% of the original oil. However, no increase was observed in a fin whale liver oil, which was of relatively high correction factor and distilled at relatively low temperature.

(2) From five to eight times concentration was attained in the most concentrated fractions.

(3) The absorption spectra of distillates showed the typical curve of vitamin A. This may mean the conversion of kitol to vitamin A and the absence or little contamination of vitamin A allied substances.

(4) The factor of distillate became remarkably higher and their ratio lower than those of the original oils. The increase ratio of vitamin A, calculated from the observed values, was higher than that from the values corrected after OSER's method.

137. Studies on the molecular distillation of whale liver oils. II. Takeshige YAMAKAWA and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. **18**, 675-682, 1953)

Several tests were carried out on the molecular distillation of whale liver oils to determine the optimum conditions in an industrial scale.

(1) The vitamin A potency of the most concentrated fractions was from 3.4 to 8.8 times higher than the original oil. On the other hand, the total amount of recovered vitamin A also exceeded over 100%, mean 150.8% and the largest 223% of the original oil, respectively.

(2) The pollack liver oil was recognized most suitable as the pick up oil.

(3) The best results were obtained with the still adopted in the following conditions, namely, two cycles circulation at 290°C in vacuum of 10^{-4}-10^{-5} mm. Hg and average current speed about 15 l/h.

VI. Forestry

Abstract Nos. 138-189

138. A brief report on the after-ripening of the seeds of *Ginkgo biloba*. Ken-ichi HATANO and Takahide KANÔ (Journ. Jap. Forestry Soc. **34**, 369-370, 1952)

When the seeds of *Ginkgo biloba* are taken in the fall (October 1st), the embryos continue growing till January in the next year at room temperature, but they cannot grow at all in about 5°C.

Seeds (at room temp. and about 5°C) germinate in 6 weeks when the germination conditions were given, and, moreover, the seeds kept at room temp. germinate more fast until 4 weeks.

139. On the amino acids in the seeds and seedlings of *Ginkgo biloba*. Ken-ichi HATANO (Bull. Tokyo Univ. Forests **44**, 221-227, 1953)

The free amino acids in the seeds, germinating embryos and seedlings of *Ginkgo biloba*, at the same time, the hydrolysate of crude proteins in the seeds, were examined by Paper Chromatography (two dimensional: Phenol-Butanol·Acetic acid).

Approximately ten amino acids were detected in all samples. It is conspicuous that tyrosine and phenylalanine were found evidently in the seedlings, and agrinine in the free state became less in the seedlings than in the seeds and the germinating embryos, and asparagine appeared after the germination. The other sulphur-containing amino acid than cystine in the crude proteins was not detected in any samples.

One unknown ninhydrin sensitive substance was detected only in all ethanol extracts, and the other unknown substance appeared in the hydrolysate of crude proteins, and the ethanol extract from the germinating embryos.

140. Forest plants of Ohshiro and Ogasa national forests, Prov. Totomi. Taizo INOKUMA and Satoru KURATA (Tokyo Rinyu, Journ. Tokyo Forestry Bureau, **5**-3, 4-10, 1952)

As supposed from the climate, the flora of the southern part of Prov. Totomi is mostly composed of warm temperate elements, as well as southern Prov. Mikawa situated in its western neighbourhood. However, it is noticeable that such southern elements as *Helicia cochinchinensis*, *Ilex purpurea*, *Meliosma rigida*, *Symplocos glauca*, *S. theophrastaefolia*, *Lasianthus japonicus*, *Lonicera hypoglauca*, *Botryopleuron axillare*, *Asplenium shikokianum*, *A. unilaterale*, *Dryopteris formosana*, *D. sparsa*, *Lindsaya Chienii*, *Plagiogyria adnata*, *Cheiropleuria bicuspe* var. *integrifolia* etc. can be seen more abundanty in the former than in the latter. As Prov. Totomi and Prov. Mikawa are situated at the eastern end of the southwestern Japan arc geologically, their florae comprize many south Japan and central China elements. Out of them, *Asplenium Yoshinagae*, *A. pseudo-Wilfordii*, *Shibateranthis Keiskei*, *Deinanthe bifida*, *Rubus pseudo-Acer*, *Cladrastis shikokiana*, *Vicia bifolia*, *Hovenia tomentella*, *Daphne kiusiana*, *Evodiopanax innovans*, *Rhododendran lateritium*, *Styrax Shiraiana*, *Abelia integrifolia*, *A. serrata*, *Pertya rigidula*, *Miricacalia Makineana*, *Chionographis japonica* etc. are found again in the Kanto Region beyond the Fossa Magna Region. Moreover, taking up the Fossa Magna Region, *Loxocalyx ambiguus* and *Rhododendron kiyosumense* are growing wild only on Mt. Nekko of Prov. Izu. These facts clearly indicate the ancient origin of these species.

141. On the distribution of *Tanakaea* in Prov. Izu. Satoru KURATA (Journ. Jap. Bot. **28**, 31-32, 1953)

Genus *Tanakaea* consists of only two species, one from Japan and the other from Central China. Like many other plants of such distribution, *Tanakaea* is considered to be comparatively ancient in its origin. In Prov. Izu, it does not occur on Mt. Amagi, a considerably recent volcano, and is nearly restricted to valleys of Mt. Nekko, more ancient. This fact that *Tanakaea* is found on geologically ancient districts is also applicable to its other habitats in Japan.

142. On some local names of *Cocculus trilobus* DC. in Japan. Satoru KURATA (Hokuriku Journ. Bot. **2**, 29-30, 1953)

In Japan, both *Cocculus trilobus* DC. and *Psychotria serpens* L. have local names meaning "to let children cry". These local names have been attributed to their two characters, inedible fruits and vines too weak to tie wood. Although only one of these two characters may have been taken into consideration when these local names

were given, these two characters have been afterwards generally thought combinec to the names. In this sense, there is no reason to restrict the etymology of a loca name to one thing, but two or more etymologies may be given.

143. Studies on *Panus stipticus* Fr. and *Trogia crispa* Fr. Hiromitsu OHASHI (Journ. Jap. Forestry Soc. 34, 220-223, 1952)

(1) The fruiting bodies of *Panus stipticus* and *Trogia crispa* and the rottec woods were observed. Considerable decay of hosts was induced by these fungi.

(2) The relation of temperatures with the mycelial growth of the fungi was studied by plate-culture method. The two fungi grew well in temperature ranging from 15°C to 30°C, and the optimum temperature for the growth seemed to lie at 25°C.

(3) In the mixed culture on peptone-glucose agar, the mycelium of *Panus stipticus* covered that of *Cortinellus Shiitake* slightly.

(4) Both of the fungi showed the BAVENDAMM's reaction positive.

(5) The test blocks of *Fagus crenata* infected with *Panus stipticus* for 10 months showed 27-38% decrease in dry-weight at the upper parts of the media, 7.3-14.0% decrease at the middle parts, and 0.2-2.0% at the lower parts. The test blocks of *Chamaecyparis obtusa* infected with *Trogia cripa* for 10 months showed 8.4-9.5% decrease in dry-weight at the upper parts of the media, 0.2-2.2% decrease at the lower parts.

144. A study on the amino acids in Shii-take (*Cyrtinellus Berkeleyana* Ito et Imai) and Masu-take *Polyporus sulphureus* Fries). Hiromitsu OHASHI (Bull. Tokyo Univ. Forests, 44, 215-220, 1953)

The amino acids in Shii-take and Masu-take were studied qualitatively by the paper chromatographic method.

Eight amino acids and unknown three ninhydrin sensitive substances were detected in the ethanol extractive of Shii-take. Twelve amino acids and an unknown ninhydrin sensitive substance in the ethanol extractive of Masu-take. And fourteen amino acids and two unknown ninhydrin sensitive substances in the crude protein of Shii-take.

145. On the separation of amino acids by paper chromatography—An introduction to its application. Hiromitsu OHASHI and Ken-ichi HATANO (Bull. Tokyo Univ. Forests 44, 229-232, 1953)

Paper chromatography to 22 refined amino acids was carried out for its application to the physiological studies of forest pathological fungi and forest tree seeds.

The chromatogram of 22 amino acids was furnished by two dimensional chromatography (Phenol-Butanol·Acetic acid), and the determination of amino acids on it was based on the Rf values and colors with ninhydrin by one dimensional, descending method.

146. On the range of attraction of the fluoscent lamps for the May-beetle. I. Masatoshi NITTO and Kanji TACHIBANA (Journ. Jap. Forestry Soc. 34, 223-227, 1952)

In connection with studies of the use of fluoscent lamps for May-beetle control, which have been made during the past two years at Sanmyoji nursery, a number of experiment have been made with the release and recovery of marked beetles. A

art of these studies reported herein, and the results summarised as follows:

(1) "Himekogane" (*Anomala rufocuprea* MOTSCHULSKY) exhibited the highest ecovery among many species of May-beetle.

(2) Of the beetles released at places 160 m or more for from the light sources r places from where the lamps were visible poorly owing to the state of topography, ery few were recaptured.

(3) The recovery percentage of the beetles liberated in windbreak-woods was lso very low, and in case of outside of them, lower.

(4) Meteorological conditions (especially, temperature, humidity, quantity of loud, phase of the moon, force of wind) always influenced upon the flying activity f the beetles.

(5) As the distance between the liberated point and a lamp increased, the reovery percentage diminished successively, showing the curve hyperbola.

(6) The majority of the beetles which responded to the light were attracted ithin 30 minutes.

147. An ecological study of May-beetles on the coastal sand dune. I. (Relaions between population of larvae and vegetational cover.) Masatoshi NITTO and anji TACHIBANA (Bull. Tokyo Univ. Forests **45**, 111-119, 1953)

Pine trees planted as windbreak on the coastal dune in the southern coast of oso Peninsula have been damaged by the larvae of May-beetles. The authors have arried out ecological studies on these beetles in order to find the effective method f their control.

An attempt was made to find the relation between population of May-beetle arva and vegetational cover on the coastal dune by belt-transact method. The results vere summarized as follows:

(1) The species of May-beetle collected in this area were Shirosujikogane *Granida albolineata* MOTCHULSKY) and Aodogane (*Anomala albopilosa* HOPE.).

(2) Higher correlation was found between population of larvae and vegetaional cover, especially of Kobomugi (*Carex macrocephala* WILLD. forma Kobomugi MAKINO), Hamahirugao (*Calystegia Soldanella* R. BR.), Onishiba (*Zoysia macrostachya* RANCH. et SAV.) and Chigaya (*Imperata cylindrica* BEAUV. var. *Koenigii* DURAND et CHINZ.).

(3) Larvae were found more abundantly in the plots having vegetational cover 1∼75%; whereas they were less in number in plots having vegetational cover near 00%.

(4) Population density of larva averaged 3.5 for Shirosujikogane and 3.2 for odogane. And its maximum value observed was 26.

(5) No difference could be observed between habitats of the two species.

148. Notes on the water relations of trees in windbreaks. Tasitiroo SATOO Tokyo Univ. Forests Misc. Inform. **9**, 25-30, 1952)

A study of water relations of trees in windbreaks was carried out under prevailng northwest wind of winter. The windbreaks studied consisted of those of 18 yearsld trees of *Chamaecyparis obtusa,* planted in single rows. Transpiration (determined y the rapid weighing method), water content of needles, and evaporation from wet lotting papers were determined on the windward and the leeward sides of the wind-

breaks. Evaporation and transpiration were higher on the windward side except when the leeward side was exposed directly to the solar radiation. Water content was always lower on the windward side than on the leeward side.

149. Seasonal change of starch content in *Quercus myrsinaefolia* and *Q. acutissia.* Tasitiroo SATOO and Takuzi TAKEGOSI (Tokyo Univ. Forests Misc. Inform. **9**, 17-23, 1952)

Seasonal change of starch content in the tissues of *Q. myrsinaefolia* (evergreen) and *Q. acutissima* (deciduous), both planted in the suburb of Tokyo, was studied. Samples from fine roots, trunks, branches, twigs, and new shoots were taken twice a month, and observed microscopically. In both spp., starch decreased suddenly in the spring after the opening of buds, but there were some differences between the two in other points. In *Q. acutissima,* starch content in roots and branches was least in early winter and spring, while it was observed only in the spring in trunks. Change in starch content was more remarkable in branches ain twigs. In *Q. myrsinaefolia* however, accumulation of starch was observed only before the opening of buds, after that it was not found until winter. Sudden decrease in starch content with the opening of buds agrees with the fact that felling of coppice trees in the growing season results in poor sprouting.

150. Relations between the rooting and the age of the tree from which cuttings are taken. An experiment using clonal materials. Taisitiroo SATOO, Ken'itiroo NEGISI and Kentarô NAKAMURA (Journ. Jap. Forestry Soc. **35**, 69-70, 1953)

Cuttings were taken from trees of *Cryptomeria japonica* of various ages belonging to the same clone, and their rooting was compared with respect to the age of the trees. Among the four clones used, rooting of clones named Zensyoo and Sigeri decreased with increasing age of trees from which cuttings were taken, but clones Nihonbare and Masumidori showed no statistically significant differences in rooting among cuttings from trees of different ages. Cuttings from adventitious branches showed higher rooting percentages than ordinary branches of the same trees, for both two trees used.

151. Response of seedlings of *Pinus densiflora* to soil dryness. Taisitiroo SATOO and Ziroo NAMURA (Journ. Jap. Forestry Soc. **35**, 71-73, 1953)

With decreasing soil moisture, transpiration rate of pine seedlings decreased at first gradually and then suddenly to a nearly constant rate which was supposed as the rate of cuticular transpiration. The sudden decrease began when soil moisture tension reached 8-9 atmospheres and the constant rate was attained when the soil moisture tension reaches near 15 atmospheres, which is nearly equivalent to the permanent wilting percentage. Water content of seedlings did not decrease until the soil moisture tension exceeds 15 atmospheres, and then decreased suddenly to the critical moisture content, and died. The critical moisture content of the seedlings seemed to be about 100% of their dry weight. The seedlings which were more resistant to soil dryness had lower top-root ratios and higher absolute weight of their tops and roots.

152. Influence of wind on transpiration of seedlings of *Cryptomeria japonica* grown under different soil moisture conditions. Taisitiroo SATOO (Bull. Tokyo Univ. Forests **44**, 1-6, 1953)

Potted one-year old seedlings of *C. japonica* were grown under three different soil moisture conditions; "wet", "moist", and "dry", and the influence of wind on their transpiration was studied, using their tops only. Though no significant difference was found between the rates of transpiration in calm air, transportation rates of them increased when they were exposed to artificial wind and the increase was the highest in those grown under the "wet" condition and the lowest in those under the "dry" condition. Water contents of needles lined up in the same order as the increase of transpiration by wind, both in the newly developed ones and in those which already had developed in the previous season.

153. Growth study of a poplar tree. Kentarô NAKAMURA and Taisitiroo SATOO (Yamanami **4**, 134-139, 1953)

Growth in height, d.b.h., volume and weight of stem were studied of a tree of poplar belonging to a clone called "Kyodai Popura". Weight, area and efficiency of leaves of it were also studied.

154. Origin and development of adventitious roots in greenwood cuttings of *Metasequoia glyptostroboides*. Seizaemon SATOO (Journ. Jap. Forestry Soc. **35**, 157-160, 1953)

Origin and development of adventitious roots in greenwood cuttings of *M. glyptostroboides* were examined. Almost all adventitious roots arose from the primary ray tissues between the outer part of newly formed xylem and phloem region, developing an elongated shape in radial direction. No callus-roots were found in this experiment. But only one root meristem was found in callus tissues as a group of meristematic cells connecting with the differentiating callus xylem.

155. On the tests of the error of a single measurement with hypsometers. Kanjirō MAEZAWA (Journ. Jap. Forestry Soc. **35**, 48-50, 1953)

(1) In the case of single measurement which has been made for 3 times replication with 2 levels of the factor of instrument, 4 levels of the factor of subject height, and 3 levels of the factor of individual, it has been cleared that the difference between instruments Asō and Weise has become highly significant.

(2) At this actual measurement, the quantity of error in sample mean for Asō is smaller than that for Weise. Accordingly it is needless to say that Asō is more accurate than Weise. Moreover Asō is supposed to be exact, because of its having been smaller in the error variance of sample.

(3) In the case of (1) the difference among individuals, with $\alpha = 0.05$, has become non significant.

(4) In consequence of 2 times replication with 4 levels of the factor of subject height and 4 levels of the factor of distance of measuring point, in the case of using Asō and Weise each, the following has been acknowledged. On Asō, both factorial effects of subject height and interaction, with $\alpha = 0.05$, have become significant. On Weise, factorial effects of subject height and the distance of measuring point, and interaction have become non significant with $\alpha = 0.05$.

(5) In consequence of 3 times replication with 3 levels of the factor of individual, 4 levels of the factor of subject height, on Christen, factorial effects of individual and subject height, with $\alpha = 0.01$, and interaction, with $\alpha = 0.05$, have become significant.

156. On the error of basal area caused by rounding. Kanjirō MAEZAWA (Journ. Jap. Forestry Soc. **35**, 160-164, 1953)

Writing the "reading" in a calliper or measuring-tape as the approximate value due to rounding and estimating a basal area (at breast-height) or gathered one, we can consider that the relative error of the estimate value caused by rounding is less than $|P_h|$ or $|P_h'|$ in the following formulas.

On single tree

(I) by calliper

$$|P_h| = \frac{12h}{\sqrt{48n}\,m}$$

P_h: Relative error

n : The number of diameter measured

m : The average diameter at breast-height from n diametrs

h : Interval of rounding in the same unit as m's

(II) by tape

$$|P_h| = \frac{12h}{\sqrt{48}\,l}$$

l : Circumference

On stand

(I) by calliper

$$|P_h'| = \frac{12h}{\sqrt{48nr} \cdot m}$$

r : The number of tree in stand

$\overline{m}$: $\sum_{i=1}^{r} m_i$

(II) by tape

$$|P_h'| = \frac{12h}{\sqrt{48r} \cdot l}$$

$\overline{l}$: $\frac{1}{r}\sum_{i=1}^{r} l_i$

According to those formulas, we can consider that, on stand where r is large, the relative error is comparatively small even though the interval of rounding is large.

Practically we can make the absolute value of relative error less than certain percentage by means of deciding h or n in above-mentioned formulas.

157. Studies on the estimate of timber volume. (1) On the basis of stratification of allocation of samples in the volume estimate of timber stands. Itizo MINE (Bull. Tokyo Univ. Forests **44**, 107-122, 1953)

As the basis of stratification or allocation of samples in the volume estimates of timber stands, diameter breast high, basal area or volume of timbers are used respectively. In the article, I have discussed which of them is the best basis to gain accuracy in volume estimates of timber stand.

In DRAUDT's method, the number of sample trees (test trees) in each diameter class bears a constant ratio to the total number of trees in the class. URICH divided the trees in a stand into a specified number of diameter groups, so arranged that each group contains the same number of trees. Then sample trees are equally divided among the different diameter groups. In these two methods, the number of sample trees in each group bears a constant ratio to the total number of trees in the class.

The most important advantage of DRAUDT-URICH's principle is that the arithmetic mean of volume of all sample trees in each group is equal to arithmetic means of volume of total trees in the stand. (DRAUDT proved above fact.)

And YOSHIDA proved that in DRAUDT-URICH's principle, the total volume of all sample trees in each group bears a constant ratio to the total volume of all trees in the group.

HARTIG arranged the trees in a stand in such a way that all groups have equal total basal areas, and allocated equal number of sample trees to each group. This method is designed to make groups in which the volume are approximately equal, but still are not exactly equal, because the heights of the trees in the separate groups are usually different.

TISCHENDORF and NEUBAUER calculated, at first, the preliminary volume of each tree in a stand from a suitable volume table, and designed to make groups in which the volume are equal. Then allocated an equal number of sample trees to each group.

Following the law of probabilities, TISCHENDORF proved that in order to satisfy the conditions to bring minimum, the sum of squares of standard errors in volume estimate, must be

$$\nu_1 : \nu_2 : \nu_3 : \ldots\ldots = n_1 m_1 : n_2 m_2 : n_3 m_3 : \ldots\ldots \qquad (1)$$

$\nu_1, \nu_2, \nu_3, \ldots\ldots$ represent number of sample trees for each group

$n_1, n_2, n_3 \ldots\ldots$,, total number of trees for each group

$m_1, m_2, m_3 \ldots\ldots$,, standard error of volume estimates for each group.

He leads after several transformations to next result.

$$m_1 : m_2 : m_3 : \ldots\ldots = l_1 : l_2 : l_3 : \ldots\ldots \qquad (2)$$

$l_1, l_2, l_3, \ldots\ldots$ represent mean velumes of sample trees for each group.

Substituting this result (2) in equation (1), he has obtained the final conclusion

$$\nu_1 : \nu_2 : \nu_3 : \ldots\ldots = n_1 l_1 : n_2 l_2 : n_3 l_3 : \ldots\ldots = V_1 : V_2 : V_3 : \ldots\ldots \qquad (3)$$

$V_1, V_2, V_3, \ldots\ldots$ represent total volume of all trees for each group.

Now I found that if we assume $m_1 = m_2 = m_3 \ldots\ldots$, then we shall have a same conclusion as DRAUDT-URICH'S principle $\nu_1 : \nu_2 : \nu_3 : \ldots\ldots = n_1 : n_2 : n_3 : \ldots\ldots$

I agree to the assumption $m_1 : m_2 : m_3 : \ldots\ldots = l_1 : l_2 : l_3 : \ldots\ldots$, but not to that $m_1 = m_2 = m_3 = \ldots\ldots$, because volume of each sample trees are widely different.

The most important condition among the above cited ones is to bring errors in the volume estimates to minimum. Therefore I conclude that as the basis of stratification or allocation of samples in the volume estimate of timber stand, volume estimated by volume table is most applicable, basal area is next, and diameters is last.

158. On the resistance of the forest-railway trolleys used in the Tokyo University Forests at Chichibu and in Hokkaido. Makoto FUJIBAYASI and Seihei KATO (Bull. Tokyo Univ. Forests **44**, 41-65, 1953)

To investigate the character and the amount of the trolley resistance on the forest-railway tracks, the authors performed various field experiments in the Tokyo University Forests at Chichibu and in Hokkaido. The explanation of the methods of the experiments, the numerical results, and the discussion about them are noted in this report. Concluding the discussion, the authors state as follows.

1. About the rolling resistance:

(a) Though the results of the push-off experiments and those of the traction

experiments are fairly coincident with each other, the latter method is superior to the former.

(b) The coefficient of the resistance in *kg/t* may be given by the formula $w_l = a + bV$; for the steel trollies (Chichibu), $w_l = 3.7 + 0.7V$, where V denotes the average running velocity in *km/hr*.

(c) The value of the coefficient for a heavy trolley is less than that of a lighter one; smaller differences appear in case of the trolleys with ball-bearings.

(d) Lubrication of the axle-bearings effects largely on the value of the coefficient.

(e) The average numerical values of the coefficient of rolling resistance at average velocity $V = 8$ *km/hr*:

For large size steel trolley with ball-bearings (Chichibu), $w_l = 8.5$ *kg/t*
For small size wooden trolley with ball-bearings (Chichibu), $w_l = 8.5$ *kg/t*
For normal size wooden trolley with plane-bearings (Hokkaido), $w_l = 13.0$ *kg/t*
For small size wooden trolley with plane-bearings (Chichibu), $w_l = 13.5$ *kg/t*

2. **About the curve-resistance:**

(a) The push-off experiments are unsatisfactory unless some special measuring instruments are devised.

(b) The values of the coefficient of the curve-resistance are not coincident with any calculated values of the ordinary formulas.

(c) For a single curve, the kinds of the axle-bearings give little effects on the value of the coefficient.

(d) It is difficult to presume the amount of the curve-resistance along a certain distance of the rail track by the individual value of each single curve-resistance. The behavior of the curve-resistance and the average value of the coefficient of it seem to depend upon the number and the length of the curves inserted between the straight lines within a certain distance of the track under consideration.

(e) The authors found that the value of the coefficient w_k is proportional to the "*Curve-factor*" or K_t of the line, which was denominated by the authors.
If K_{lt} denotes the ratio

$$\frac{\text{Total sum of length of curves, the radius of curvature of which are less than } i\, m}{\text{Total length of the line}}$$

and K_{nt} the frequency of curves within one *km* of the line, then $K_t = K_{lt} \times K_{nt}$. For the steel trolleys (Chichibu) w_k is given by the formula $w_k = 1.05\ K_{25}$, when the average velocity $V = 4.2 \sim 5.5$ *km/hr*.

(f) In general, the coefficient may be given by the formula $w_k = \alpha K_t$.

3. **About the practical coefficient of resistance:**

(a) The average value of the practical coefficient of resistance for the steel trolleys (Chichibu) $w_p = 19$ *kg/t*, when average velocity $V = 4.6$ *km/hr*.

(b) Such high value, 2.75 times larger than the value of the coefficient of the rolling resistance w_l, indicates the characteristics of the railway lines in the ragged mountain forests.

(c) In general, the average value may be given by the formula $w_p = w \mp s^0/_{00} = w_l + w_k = (a + bV) + \alpha K_t$.

4. **About the start-resistance:**

(a) The amount of the start-resistance appears to be much irregular.

(b) Though the maximum value of the start-resistance is very high, it occurs only instantaneously and seems to bring little effect upon the practical problems.

159. Management of the logging operation. Seihei KATO (302 p. Asakurashoten, Tokyo 1952)

The logging operations are playing important roles in the forest economy. Very wise and scientific management of the operations should be emphasized to promote the rationalization of forestry increasing the timber productivity of the forests in the limited area of this country. The author applied the theories and the principles of the scientific management of modern productive industries to the field of the logging operations.

In the first place, so-called "Break-even theory" of the cost-control and the principles of the cost-analysis are applied to each stage of the operation, such as cutting timbers, log-making, log-hauling, transportation etc. For the purpose of radical improvement in the economical mechanization of logging, the nature and the amounts of the fixed costs, the depreciations, the machine rates and the operation-costs are specially discussed. In the field of the log-transportation, the author predicts the great progress of the motor-truck hauling in the near future, which will replace the forest railways in the trunk lines of transportation. The problems of the forest roads, especially the leading principles of the road system planning and the relations between the behaviours of the roads and the hauling efficiencies are investigated on the stand points of engineering and economy.

In the second place, the efficiencies and the costs measured in the various methods of the logging operations are investigated. The investigation was based on the numerous data gained from the results of the actual operations performed by the National Forest agencies and other private logging operators in the various regions of this country. The comparative studies are also made between the old and the new kind of the operations; the power-saw cutting, the mono-rail-sledge hauling, machine skidder and the skyline cable hauling, the motor-truck and the tractor hauling, the minor railway and the cable-railway hauling, the mechanized timber-yard works, and the mechanized earth works are included in the new kind.

In the third place, as an appendix, the author compiled an illustrated list of the modern logging machines prepared by the Forestry Mechanization Association.

160. Wooden beams prestressed by eccentric tensioning. Seihei KATO, Minoru KAMIIZAKA (Trans. 61st Meeting, Jap. Forestry Soc., 191-192, 1952)

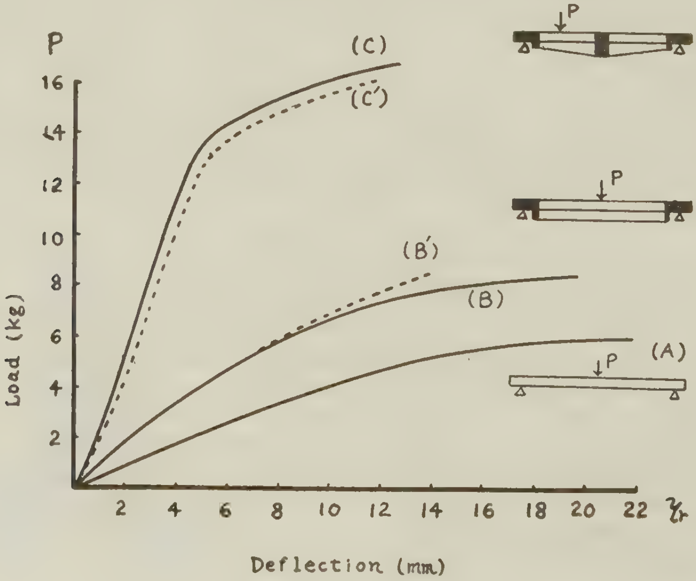
The mechanical properties of the prestressed wooden beams, which were originally designed by KATO, were tested and compared with the simple beams under single concentrated vertical loadings. Two types of the beams, prestressed by eccentric tensioning, were used for the experiment, the dimensions of the test-pieces and the result of the bending tests are shown in Table 1 and 2. The behaviors of the prestressed beams were observed to be similar to those of the fixed beams or the continuous beams with both ends fixed. The flexual rigidity and the strength of them were proved to be considerably higher than those of the simple beams. These general tendencies are shown in the figure.

Table 1. Test-pieces.

	Types	Wood species	Moisture content	Cross-section	Effective span for bending test (l_0)	Inclination of tension wire	Pre-tensioning load (Q)	Initial reverse deflection at span center (η_0)
A.	Simple beam (Comparison)	*Chamaecyparis obtusa* S. et Z.	Av. 18%	6×10*mm*	220*mm*	—	0 *kg*	0 *mm*
B.	Prestressed beam with a parallel tension wire	,,	,,	,,	,,	0°	a. 5.0 *kg* b. 2.5 *kg*	3.0 *mm* 1.0 *mm*
C.	Prestressed beam with inclined tension wires and a central strut	,,	,,	,,	,,	1.5°	2.5 *kg*	1.0 *mm*

Table 2. Result of the bending tests.

Types and pre-tensioning	Relative center deflection η_r (*mm*): Vertical load P 1 *kg*	3 *kg*	5 *kg*	10 *kg*	Ultimate load (*kg*)	Relative deflection at the stage of rupture (*mm*)	Modulas of elasticity (*kg/cm*²)
A.	2.4	6.0	11.6	—	6.3	21.7	6.8×10^4
B. a.	1.4	2.9	5.4	—	7.4	16.7	15.9×10^4 (Nominal modulus at $P=3\,kg$)
b.	1.2	3.4	6.3	—	8.5	20.8	15.7×10^4 (,,)
C.	0.6	1.3	2.1	3.6	16.5	12.5	35.0×10^4 (,, $P=10kg$)



161. The vibration method of tension-measurement for skyline cables and its application. Seihei KATO and Masakazu MARUYAMA (Journ. Jap. Forestry Soc. **35**, 59-61, 1953)

(1) The vibration method of indirect tension-measurement for any existing skyline logging cable is proved by the authors' experiments to be very convenient and considerably accurate.

(2) The values of cable tension measured by the propagating velocity of the vibration waves may be utilized for practical purposes in many cases.

(3) Probable maximum tension, $Tmax$, of a skyline cable, to which a single concentrated load is applied, is given by the formula,

$$Tmax = \gamma \cdot T$$

where, T denotes the value of tension of the same cable measured by vibration method when no load is put on it. Coefficient γ is theoretically calculated according to the form and the weight of the cable and the amount of the load put on it.

162. Theoretical analysis of the vibration method presuming the maximum tension of a skyline cable. Seihei KATO and Masakazu MARUYAMA (Bull. Tokyo Univ. Forests **44**, 67-81, 1953)

It is an important procedure to presume the maximum tension of a skyline wire-rope on which any load is put, especially in case of determining the factor of safety and the allowable maximum load of an aerial cableway or a skidding skyline used for logging operation. The authors investigated the vibration method of measuring cable-tension by some field experiments, and theoretically analysed the existing relations between the tension of an unloaded cable and that of the same cable on which a load is put. A brief summary of the results is noted as follows.

(1) The tension (T) of a skyline cable, unloaded, is given by the formula,

$$T = \frac{p}{9.8} \cdot v^2 = \frac{p}{9.8}\left(\frac{2L}{t}\right)^2$$

when the propagating velocity (v) of the vibration-wave of the cable is measured directly. The measurement of v may be practically performed by a stop-watch with sufficient accuracy.

(2) The ratio (γ) of the maximum tension ($T_P max$) of a skyline cable, with any single concentrated load (P) put on it, to the average tension (Tav) of the same cable with no load is theoretically given by the following formulas which were derived from the theory of the parabolic cable.

$$Tav = pL \cdot \frac{C}{A} = C \cdot T_m$$

$$T_P max = pL \cdot \frac{D}{A}$$

$$= \frac{pL}{8s\cos\alpha}\left[a + \left(b\frac{\tan\alpha}{2s} + c\right)16s^2\cos^2\alpha\right]^{\frac{1}{2}}$$

$$\gamma = \frac{T_P max}{Tav} = \frac{D}{C}$$

where p = weight per unit length of cable

C, A = coefficient relating to α, s

L = length of cable

l = span length

$w = pL/l$

s = sag ratio of span (at the center of the unloaded cable)
n = load ratio (the ratio of the applied load, including weight of the carriage, to the own weight of the cable between the both supports.)
α = inclination degrees of the straight line connecting lower and upper supports of the cable
$a = 1 + 3(n + n^2)$
$b = (1+n)\{1+3(n+n^2)\}^{\frac{1}{2}}$
$c = (1+n)^2$

(3) Then, the maximum tension ($Tmax$) of a loaded cable can be presumed by the value of the tension (T), measured by the vibration method with no load, multiplied by a certain coefficient (γ), as shown in the formula

$$Tmax = \gamma \cdot T$$

(4) The numerical values of the coefficient (γ) are compiled in a table for various probable values of s, α and n, which may be practically utilized for presuming the maximum tension of any skyline cable.

163. Inspection of wooden beam bridges for their strength and durability. Masakazu MARUYAMA (Bull. Tokyo Univ. Forests **44**, 83-106, 1953)

Numerous wooden bridges built for forest-roads and forest-railtracks of this country can hardly keep their average lives of more than 8～10 years of age after their erection; they are forced to be continuously repaired or reerected year after year. Decaying or decrease of the strength of timber is the chief reason of shortening the lives of such wooden structures. This distinguished fact throws before us the important problems how to inspect the strength of existing wooden bridge-structures, how to recognize their accurate bearing capacity for any assumed loading at present stage, and how to judge their future durability.

Aiming to solve these problems, the author at first discussed theoretically about the strength and the durability of wooden structures in general, then carried out field experiments with beam-bridges built for the minor forest-railway tracks of the Tokyo University Forest at Chichibu in Saitama Prefecture. The bridges were mainly of *Tsuga* wood (*Tsuga Sieboldii* CARR.) and the effective spans of beams were 3～4 *m*.

Following three methods were chosen for the experiments:

(1) Plunging test—indirect determination of the strength of the construction members (beams) of the existing bridges by means of a handy testing instrument specially devised for the purpose. The instrument is placed and operated on the surface of a wooden member, and the plunging depth of a cylindrical rod attached to the instrument is measured, and by which the bearing strength of the member is judged.

(2) Deflection test—investigation of the flexual rigidity of main beams by means of direct measurement of maximum deflection of them under a certain actual load statically applied.

(3) Dynamic test—examination of the impact effect of an actual live load on main beams by means of the direct measurement of deflection. Maximum deflection of each beam of a bridge when the load is fixed at the span center and when the load is moving at normal speed, are both measured independently and compared.

A brief summary of the results is noted as follows:—

(1) It was found by the plunging tests that the breaking strength of the tested

part of the member was less than 60% of that of a fresh timber when the plunging depth of the instrument was more than 4 *mm*. Factor of safety of the wooden bridge-beam for an assumed 4 *t* locomotive load was calculated at average 3.3 when probable defects in timber and long-time-loading effect were taken into account. Accordingly, factor of safety of the beams, on many parts of which plunging depth of more than 4 *mm* appears, might be less than 2.0. The bridge including these beams is, needless to say, at the last stage of durability and already dangerous. Strength of a bridge can be judged with considerable accuracy by this plunging test.

(2) Usual specification for construction of wooden bridges requires that the maximum deflection of a beam should be less than $l/300$, denoting l for effective span of the beam. Measured amount of the deflection, under an actual 4 *t* locomtive load, of the beams which are in early stage of duration after construction were about one-third of this allowable limit and fairly equal to the values mathematicaly calculated by the formulas of elastic theory. Deflection of beams, which were more than 8 years old after construction and strength of which indicated by the plunging test being at the limit of safety, appeared nearly equal or slightly larger than the values of allowable limit above mentioned. This means that the rate of decrease in flexaual rigidity (EI) of such beams is more than 70%. Therefore, any beam of *Tsuga* wood (effective span less than 3 *m*), actual deflection of which approaches to the allowable limit of the specification ($l/300$), should be understood that it is already at dangerous stage even if there is no remarkable change on its superficial appearance. Thus the method of deflection test is proved to be very useful and practical.

(3) Impact coefficient is usually taken at 0.3 refering to the weight of an assumed live load in the designing practice of our forest railway bridges. Comparative studies on the result of the dynamic test proved that, though the impact effect of the actual live load was about 10% of statical effect of the same load in case of bridges of younger age, it reached higher value (30∼50%) in case of aged bridges which were judged as dangerous by the preceding tests, i.e. plunging depth of the testing instrument on main beams more than 4 *mm* or statical deflection larger than $l/300$. Therefore, this third method is also effective and practical for the judgement of durability of a wooden bridge.

164. Erosion control of Japan. Sadao OGIHARA (Tokyo Univ. Forests Misc. Inform. **9**, 31, 1952)

Beginning with the importance of erosion control in Japan, the first chapter explains the history, present executive authorities, and education and research. In the following chapters the author develops erosion situation and methods of control. Further he expresses his opinion about "How the erosion control should be in the future?", emphasizing the function of forest from standpoints of both land preservation and economic.

165. Calculation of discharge from triangular notch. Isao YAMAGUTI (Tokyo Univ. Forests Misc. Inform. **9**, 79, 1952)

For calculation of stream flow from water level curve, the step method has been the only one to be applied.

In the step method water level curve is divided into such small parts that each

part may be regarded as a straight line.

Then, for each part, either of the following two methods is applied to get stream flow,

method 1. $Q=f\left(\frac{h_1+h_2}{2}\right)T$ method 2. $Q=\frac{f(h_1)+f(h_2)}{2}T$

where, $Q=$ Stream flow during a given time interval T in which water level curve is regarded as a straight line,

$h_1=$ Water level at the begining, $h_2=$ Water level at the end.

From exponent of water level, we know that method 1 always gives results too small while method 2 always gives results too large.

In case of triangular notch for which the relation between flow intensity q and h is given as $q=ch^{\frac{5}{2}}$, the error of calculation is more enlarged.

The writer has made comparison between the values calculated by the method 1 and 2, and those by the theoretically correct formula.

It is judged that the methods 1 and 2 are unsuitable.

The writer made a computation improving method 1, 2 with diagram.

More accurate calculation of stream flow is to be carried out by it.

166. On the difference in cutting action between the circular rip-saw and cross-cut saw. Hisashi FUKUI (Trans. 61st Meeting, Jap. Forestry Soc., 218-220, 1952)

About the flat grain board of "sugi" (*Cryptomeria japonica* D. DON) the effects of the angle between the direction of feed and that of the grain and the feed speed on the power of cutting by the circular rip-saw or cross-cut saw have been studied in order to find out some differences in the cutting action between these two kinds of the saw. The power required is measured by wattmeter.

From the results obtained I have led the following conclusion.

In the higher range of feed speed, as it is said usually, the cross-cutting by cross-cut saw and the ripping by rip-saw are far easier than that by rip-saw and this by cross-cut saw, respectively, but in the lower range (0∼8 m/min.), though the ripping by rip-saw is easier than by cross-cut saw, it is contrary in the cross-cutting, that is, the cross-cutting by rip-saw is also easier than by cross-cut saw.

167. Studies on plywoods. X. Warping of plywood in relation to the condition of core-veneer. Shinji HIRAI, Takashi SHIMIZU and Naoyoshi TAKUWA (Wood Industry 7, 393-397, 1952)

The warping of the plywood increases as the moisture content descends in the process of natural or artificial drying, and it is divided into two kinds, which are the cupping and the twisting.

An experiment which measured the degree of cupping and the twisting numerically on 3-ply 6 mm plywoods of "buna" (*Fagus crenata* BL.) indicated the following results.

The plywood which has thicker core of 2/3 of total thickness (1·4·1) indicates small cupping compared with that of the composition (2·2·2). When the moisture content of the core is under 10%, the value of the twisting is low in the one (1·4·1), and when over 18%, that is in the other (2·2·2).

In both cases of the composition of ply, the degree of warping is exceedingly

small when the moisture content of the core is under 10% and when it has smaller cutting checks.

168. Studies on laminated woods with sawn boards. I. Preparation of a laminated wood of *Cryptomeria japonica* D. Don. Shinji Hirai, Hisashi Fukui and Saburô Shibahara (Bull. Tokyo Univ. Forests **44**, 123-138, 1953)

By gluing seven pieces of the flat grain board (25 mm in thickness) of "sugi" heart wood (*Cryptomeria japonica* D. Don) with room-temperature-setting urea resin adhesives containing filler (wood flour), we have manufactured one laminated straight timber of 2,000 mm length, 100 mm width and 175 mm thickness. Each board is 14% in moisture content. Assembling pressure is 5 kg/cm^2 and measured by spring gauge. Two of six glue joints in this laminated timber are composed of the surfaces machined with super-surfacer (fine resurfacing machine), another two of them of the surfaces machined with surface planer and the remainder of the surfaces machined with band saw only.

On this product the influences of the clamps used to apply pressure, the surfacing quality (roughness) of the board to be glued and the defects on the adhesive strength have been studied. The results obtained are as follows:

(1) Though the adhesive strength varies in the direction of length irregularly, the influence of the clamps on the adhesive strength is not recognized.

(2) As to the adhesive strength of the glue joint bonded by such urea resin adhesives containing filler as used in this fabrication, the value for the surfaces machined with band saw only may be larger than that for the surfaces machined with super-surfacer (significant within 0.1% level), and there may be no significant difference between the surfaces machined with super-surfacer and those machined with surface planer.

(3) Particularizing the two glue joints of the surfaces machined with super-surfacer, the adhesive strength and the wood failure percentage of the glue joint which has been spread with larger amount of glue (793 g/m^2) are higher than those of the other (293 g/m^2) (significant within 2% level).

(4) There may be no difference of adhesive strength between the glue joint of the part of normal straight grain and that of cross grain (Crossing angle (θ) to the glue line is $10° \sim 20°$).

(5) The value of wood failure percentage is larger in the glue joint of the surfaces machined only with band saw than in that with super-surfacer or surface planer. This shows the same tendency as in the value of adhesive strength. As the cause of this fact, it can be thought that the larger the roughness of the surface to be glued is, the more the mechanical adhesion may be strengthened, and it becomes larger than the shearing strength of the wood.

169. Wood study of *Larix leptolepis* Gordon from an artificial stand in the Tokyo University Forest at Yamanaka in Yamanashi Prefecture. Shinji Hirai (Bull. Tokyo University Forests **44**, 139-151, 1953)

The physical and mechanical properties of the wood of "karamatsu", *Larix leptolepis* Gordon, from an artificial stand in the Tokyo University Forest at Yamanaka in Yamanashi Prefecture were studied by the standard method of testing wood in Japan. The average values of several properties are shown in the following table.

Property	Average value
Average ring width	4.2 mm
Moisture content	14.3%
Specific gravity	
Air dry	0.47
Oven dry	0.41
Compression parallel to grain	
Maximum crushing strength	313 kg/cm^2
Total compression perpendicular to grain	
Proportional limit	44 kg/cm^2
Partial compression perpendicular to grain	
Proportional limit	85 kg/cm^2
Stress when indentation is 10% of height of specimen	112 kg/cm^2
Tension parallel to grain	
Maximum tensile strength	644 kg/cm^3
Bending	
Modulus of rupture	614 kg/cm^2
Modulus of elasticity	5.7×10^4 kg/cm^2
Shear parallel to grain	
Maximum shearing strength	76 kg/cm^2
Splitting parallel to grain	
Splitting resistance	26 kg/cm
Impact bending in CHARPY'S type	
Absorbed energy	0.41 kgm/cm^2
BRINNELL hardness	
End face	4.95 kg/mm^2
Edge grain face	2.21 kg/mm^2
Flat grain face	2.20 kg/mm^2
Average shrinkage per 1% moisture content from air dry to oven dry based on dimensions when 15% of moisture content	
Tangential	0.239%
Radial	0.098%
Longitudinal	0.029%

170. On a simple testing apparatus of YOUNG's modulus in bending test. Kakuichi KITAHARA (Bull. Tokyo Univ. Forests **44**, 153-157, 1953)

The author designed a new simple apparatus of measuring YOUNG's modulus in bending of wood. The principle of it is as follows (Fig. 1): EAB is a cantilever and its length is 200mm and its thickness, h, is 3 mm. ADC is a circular arc, made of iron and its radius is R, 200 mm. Load P is hanged at B. In ordinary cantilever, $l/\rho = M/EI = Pl/EI$. Where l is AB, ρ is radius of curvature, M is bending moment, E is Young's modulus and I is moment of enertia. When the load is larger than EI/R, $E = PRx/I = 12PRx/bh^3$. Where x is DB. Then x is measured, and E is able to be calculated. If h, R and P are decided adequately, the value of x is larger than 100 mm. So x is measured by mm scale, and the error is smaller than 1%.

The values of this test coincide with those measured by ordinary cantilever method, but are smaller than those measured by two points support and center load method.

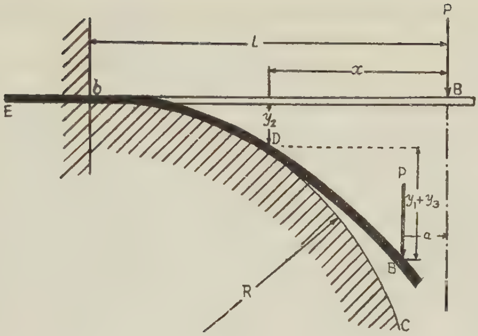


Fig. 1 Principle of the apparatus

171. On the relation of boiling condition and compressive deformation concern-ng wood-bending. Yoshio SAITO, Kakuichi KITAHARA and Shyoji YAMAZAKI (Trans. 3th Meeting Jap. Forestry Soc., 225-226, 1952)

The tested woods were "Mizunara" (*Quercus crispula* BLUME), "Yachidamo" *Fraxinus mandshurica* RUPR.) and "Keyaki" (*Zelkowa serrata* MAKINO).

When the boiling time is prolonged, keeping the temperature, 98°C, constant, he s-s diagrams incline to lower. But it is too long, and wood becomes brittle and naximum load decreases.

When the temperature of heat water is changed, keeping the boiling time, 50 ninutes, constant, s-s diagrams incline to lower. The rupture types scarcely change n each temperature. The purpose of softening wood is achieved more easily by igh temperature.

172. A fundamental study on the drying conditions of wood-bending. Yoshio SAITO, Kakuichi KITAHARA and Seiichi KANO (Wood Industry 7, 440-443, 1952)

The purpose of drying bent wood at its form is to minimize the spring-back when t is dismantled from the form and to reduce its water content. When bent wood s dryed, it is heated on severe conditions. And in this case, the factors to minimize he spring-back are water content at the time of dismantling, temperature and time t the form. In this test the spring-back is defined as follows, $A = (1\text{-}6\rho) \times 100\,(\%)$. Where ρ is radius of curvature and 6 is the radius of the form (cm). In this test hey executed following experiments: (1) The relation between spring-back and he time after dismantling from the form, changing water content and keeping time nd temperature constant. (2) The relation between spring-back and time after ismantling from the form, changing temperature and keeping time and water content onstant. (3) The relation between spring-back and time after dismantling from he form, changing time at the form and keeping temperature and water content onstant. (4) The relation between edge-grain bending and flat-grain bending on he same conditions of (3). The obtained results are as follows: (1) Water con-ent at the time of dismantling from the form should be lower than 10%. (2) Tem-erature should be higher than 80°C. (3) Time at the form scarcely has influence n the spring-back. (4) Spring-back of edge-grain bending is larger than that of at-grain bending.

173. Creep of wood beam in moisture-transitional state. Yasushi SUZUKI (Trans. 61st Meeting Jap. Forestry Soc., 210-211, 1952)

Creep of wood beam in moisture-transitional state such as in absorption or desorption process was studied. Test results show that creep rate of beam in such state is much greater than that in equilibrium state even if the moisture distribution and resulted internal stress are taken into account. For instance, creep of beam expressed by α_4, the ratio of the beginning 4 minutes creep to elastic deformation, in moisture absorption process from oven-dry to air-dry condition is much greater than that of longly air-dried beam. Such striking difference in creep rate is also found between newly equilibrated specimen to any moisture content and longly equilibrated one in the same moisture content. Reason of these facts remains unexplained. If the similar tendency is discovered in compression creep which is so difficult to measure that any satisfactory experiment has not yet been performed, the above said facts can be attributed to any microstructural phenomenon of wood.

174. Studies of bamboo. IX. Water relations of bamboo. Yasushi SUZUKI (Bull. Tokyo Univ. Forests **44**, 159-186, 1953)

Water-soluble extractives contained in bamboo (*Phyllostachys pubescens* MAGEL) are highly hygroscopic and have considerable effects on the physical properties of bamboo. Each cell cavity of green bamboo is almost saturated with sap throughout all seasons. This is probably due to osmotic pressure caused by semipermeable membrane and concentration of water-soluble extractives in sap. As the result of water saturation of cell cavities, green bamboo is apt to collapse by liquid tension in drying. Drying rate in seasoning is more restrained than extracted wet bamboo and native green wood, chiefly because of the lowering of vapor pressure caused by concentration of extractives in sap. Vapor pressure drop also affects on the isothermal sorption curve. The almost all the part of bamboo culm adsorbs moisture far more than ordinary wood in humid air.

Shrinking which occurs in the region of less than 15% moisture content is normal dimensional change by decrease of bound water in cell wall and shrinkage versus moisture content relation holds linearity. Mechanism of this shrinking is, however, considered to be somewhat different from that of wood. Parenchyma tissue shrinks very small compared with wood, while vascular fiber shrinks as large as wood fiber or tracheid of the same specific gravity. Therefore, shrinkage of bamboo, especially of inner parenchyma-rich part, is smaller than that of wood of the same specific gravity. The reason of this unusual small shrinkage of parenchyma tissue is supposed to be due to peculiarity of its cellular structure.

Fiber saturation point of extracted piece of outer fiber-rich part is about 5% moisture content or lower than those of extracted piece of inner part and of wood. Such low hygroscopicity of bamboo fiber may be explained from its well arranged microstructure.

175. On the determination of moisture content of wood under high-frequency drying process. Takashi YAMAMOTO (Trans. 61st Meeting Jap. Forestry Soc., 200-201, 1952)

This paper deals with the determination of moisture content of wood under high-frequency heating, without cutting out the specimen from the wood.

Moisture content M% is calculated from the formula,

$$\frac{M}{100}=\frac{3600\,\eta W}{4.2\,g\theta}-0.327$$

W : Total input power of high-frequency generator (kWh)
g : Weight of wood under drying (kg)
θ : Temperature raise of wood under drying (°C)
η : Efficency of high-frequency generator

This method is now applied in drying the columns (*Chamaecyparis obtusa* ENDL., ameter ca. 80 cm., length ca. 500 cm.) of Horyuji Temple.

Summarily, this method is based on the heat capacity of wood and of water, nsequently, there is no fluctuation caused by difference of species and specific avity of wood.

176. Phenol oxidases in the wood-rotting fungi. I. On reaction of oxidase with e phenolic compounds. Toschio FUKUZUMI (Journ. Jap. Forestry Soc. **35**, 139-141, 53)

It was observed that a colour was produced on the agar containing phenolic bstances by phenol oxidases secreted from the mycelium of the wood-rotting fungi, d it was recognized that there were somewhat different function and substrate ecifity in accordance with different species of the fungi, and alpha-naphtol was perior agent to tannic acid for the purpose of facilitating isolation of white rot ngi.

The enzyme preparation of phenol oxidases was made from the mycelium of *ereum umbrinum,* and it was investigated that the purple precipitate was produced om alpha-naphtol solution by the function of the preparation of phenol oxidases.

177. On the formation of pellets and their respiration of the wood-rotting fungus *olystictus sanguineus.* Toshio FUKUZUMI and Takafusa HARAGUCHI (Journ. Jap. orestry Soc. **35**, 172-174, 1953)

The minute pellets of mycelium of Polystictus sanguineus were gained by shakg culture. In this case, mycelium used for a inoculum was necessary to be young, nd the shaking rate must be kept at 130 cycles a minute. Growth of the pellets eached to almost maximum on the third day of incubation. The Qo_2 varied remarkably uring culture, the peak of which was observed also on the third day. The pellets eing starved, Qo_2 fell to around 15. Endogenous respiration was unaffected by the H between 4.4 and 8.0. But the rate of O_2 uptake decreased slightly at high pH, hen glucose was added as substrate.

178. Studies on ashes in fungi. Inosuke IWADE and Maki SAWADA (Journ. Jap. orestry Soc. Transactions 266-267, 1952)

The following 25 elements (metalic elements 17, non metalic elements 8) were ound in ashes of fruit-bodies of 112 species of fleshy fungi and 13 species of hard ungi (leathery, corky and woody): H, C, N, O, Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, ', Cr, Mn, Fe, Ni, Cu, Zn, Mo, Ag, Sn, and Pb.

The ashes of fleshy fungi were generally perti-coloured, deliquescent, and crystalne, and those of hard fungi were light coloured amorphous and non-deliquescent. 'his difference is presumably attributed to the difference of contents of components

in ashes: that is to say, in fleshy fungi, K_2O: 57.85%, P_2O_5: 18.30%, CaO: 1.14%, in hard fungi, K_2O: 26.41%, P_2O_5: 12.33%, CaO: 18.15%.

179. On constituents of fruit-body of *Fomes pinicola* I. Takeo SHIBAMOTO Kyoji MINAMI and Toshio TAJIMA (Journ. Jap. Forestry Soc., **34**, 390-392, 1952).

From the alcoholic extract of the fruit-body of *Fomes pinicola* FR. fungus cerebrin, trehalose, ergosterin were isolated.

180. On constituents of fruit-body of *Fomes pinicola* II. Takeo SHIBAMOTO Kyoji MINAMI and Toshio TAJIMA (Journ. Jap. Forestry Soc., **35**, 56-58, 1953).

From the ether-extracted residue of alcohol extract of fruit-body of *Fomes pinicola*, two substances, A (m.p. 260-263°C (decomp.), $[\alpha]_D^{15} = +8.6°$ (in pyridine)) and B (m.p. 305-308°C (decomp.), $[\alpha]_D^{20} = +43°$ (in pyridine)) having their formula as $C_{30}H_{46}O_4$ were isolated. They have one double bond and one carboxyl group, and are likely to belong to triterpenoid on color reactions. In A one carbonyl group is ascertained as 2,4-dinitrophenylhydrazone, but from B any carbonyl derivative is not obtained. A gives a methyl ester, m.p. 181-181.5°C, $[\alpha]_D^{9} = +20.8°$ (in chloroform) and a monoacetate, m.p. 177-180°C, and B gives a methyl ester m.p. 178-178.5°C $[\alpha]_D^{7} = +44°$ (in chloroform) and, as an acetate, a needle crystal m.p. 160-162°C though its formula is corresponding to $C_{32}H_{46}O_8$.

181. The influence of aeration of the water-culture of *Cinnamomum Camphora* SIEB. (III). The influence of hydrogen peroxide addition to the medium upon the growth of seedlings of *Zelkowa serrata* MAKINO in solution culture. Takeo SHIBAMOTO and Akira KAWANA (Bull. Tokyo Univ. Forests **44**, 23-29, 1953)

Experiments were made on the relation between the growth of seedlings of *Zelkowa serrata* MAKINO in solution culture and the concentration of hydrogen peroxide added to the nutrient substrates.

The amount of oxygen absorbed and that of dissolved oxygen required by the seedlings vary depending on the season and the temperature, and their influence on the growth of the seedlings is not negligible. When supplied with an excessive amount of hydrogen peroxide, the plant exhibited oxygen toxicity, but those series with a proper amount of it showed favorable results on the growth of roots and leaves.

The aeration by addition of oxygen peroxide to nutrient solution is considered convenient and effective.

182. Studies on the so-called Boucherie process for wood poles preservation by pentachlorophenolates. Takeo SHIBAMOTO and Ryuji SHOJI (Bull. Tokyo Univ. Forests **44**, 187-199, 1953)

The authors studied on some properties of the pentachlorophenolates and so-called Boucherie process for wood pole preservation.

(1) The pentachlorophenolates are soluble in water and valuable fungicide on the agar medium but their treated wood were easily attacked by wood destroying fungi, because their penetration into wood tissue is very poor under usual conditions.

(2) By Boucherie process, Na- and NH_4-PCP were penetrated satisfactorily for sap wood of Sugi (*Cryptomeria japonsa* DON) and Hinoki (*Chamaecyparis obtusa* SIEB. et ZUCC.), and 1.3 kg. cubic meter is very valuable for preservation, but 0.4 kg is not effective.

(4) The penetration velocity is greater at high concentration than at lower ıcentration, but in both cases, it is smaller than that $CuSO_4$.

(5) The amount of Na-PCP leached out by water is far smaller than that of SO_4, and retreatment by $Al_2(SO_4)_3$ is very suitable.

(6) From these results, pentachlorophenolates will be able to be used well for ucherie process, but they must study further on the optimum treatment conditions.

183. Composition of barks of birches in Hokkaido. Takeo SHIBAMOTO, Kyoji NAMI, Tomohiro MASUTANI and Sumiko KUBOTA (Bull. Tokyo Univ. Forests, **44**, 1-204, 1953)

Using analytical methods for wood, the compositions of outer bark of *Betula lati-ia* KOM., *B. Maximowicziana* REGEL and *B. Ermanii* CHAM. var. *communis* KOIDZ. ›wn in the Tokyo University Forest in Hokkaido were determined. The amount alcohol-benzene extract decreased in the cited order, and, reversely, those of 1% OH extract, CROSS-BEVAN cellulose and KLASON lignin increased in the same order. e methoxyl content, in any case, was in trace and this throws doubt on the ex-ence of lignin in those outer barks.

184. Studies on Todomatsu-balsam. III. Some observations on the properties rosin of Todomatsu-balsam. Takeo SHIBAMOTO, Kyoji MINAMI and Masayuki AMADA (Bull. Tokyo Univ. Forests **44**, 233-235, 1953)

The solubility of the rosin of Todamatsu-balsam was determined.

185. The antipulping effect of the flavanonol of larch heartwood in the sulphite ok (1). Nobuhiko MIGITA, Junzo NAKANO and Taiji IOROI (Journ. Jap. Tech. Assoc. ılp Paper Ind. **5**, 399, 1951)

J. C. PEW (1949) has isolated a crystalline compound, the 3, 3′, 4′, 5, 7-penta-ydroxyflavanone, from the heartwood of Douglas fir, and recognized that owing the presence of the flavanonol the wood is hardly pulped by the sulphite cook. Re-ntly M. HASEGAWA (1951) has identified the flavanonol of the larch heartwood with at of the Douglas fir heartwood. In this paper, the antipulping effect of the flavanol the larch heartwood in the sulphite cook was examined.

The sulphite cooks were carried out on the following samples; four kinds of e larch heartwood in which content of the flavanonol varied, the larch heartwood tracted with methanol (the flavanonol is soluble in methanol), the spruce wood, the ruce wood impregnated with the flavanonol and with some compounds related to the emical structure of the flavanonol such as pyrocatechol, resorcin, phloroglucin and econic acid. The results of cooks indicate that the flavanonol has the antipulping fect in the sulphite cook of the larch heartwood.

186. The antipulping effect of the flavanonol of larch heartwood in the sulphite ook (2). Nobuhiko MIGITA, Junzo NAKANO, Isamu SAKAI and Shoichi ISHII (Journ. ap. Tech. Assoc. Pulp Paper Ind. **6**, 476, 1952)

In the previous report the authors concluded that the flavanonol in larch heart-ood has the antipulping effect in sulphite cook owing to the condensation of flavanonol nd lignin. Some studies were carried out on the antipulping effect of larch heart-ood in sulphite cook, especially the behavior of flavanonol and the condensation of gnin and flavanonol during the cooking. The results are summarized as follows:

(1) The occurrence of the condensation product was not remarkable in th cook of isolated native lignin and flavanonol.

(2) Quercetin (yield, over 65+) was obtained by the sulphite cook o isolated flavanonol. A large amount of quercetin (yield, over 0.5% based upon th larch heartwood) was also obtained from the waste liquor in normal sulphite cool of larch heartwood.

(3) The yellow color of pulp obtained by sulphite cook seems to be due t the presence of quercetin. The result of the determination with ultraviolet absorp tion spectrum indicates that the content of quercetin in the yellowish pulp is les than 0.12%

187. The occurrence of α- and β-thujaplicin in the essential oil from the woo of *Thuja occidentalis* L. Tomoichiro NAKATSUKA (Journ. Jap. Forestry Soc. **34** 248-250, 1952)

The acidic portion of the essential oil prepared from the wood of *Thuja occi dentalis* L. planted in Hokkaido was examined, α- and β-Thujaplicins were found i the acidic portion, the former is the major constituent of the acidic portion and th latter is the minor one.

188. Experiments on viscose wood adhesive. Tomoichiro NAKATSUKA and Chu zaburo WATANABE (Journ. Jap. Forestry Soc. **34**, 307-309, 1952)

(1) It is preferable to use bleached pulp of high quality for the preparatio of viscose wood adhesive.

(2) Although the viscosity of viscose is depressed to some extent by using th mercerization liquor which contains sodium sulphide, the degree of depression of vis cosity is not sufficient to use non-aging-alkalicellulose. The joint strength of viscos adhesive is depressed by using the mercerization liquor containing sodium sulfide. I case of using non-aging-cellulose, the control of the viscosity and the strength o the adhesive may be attained by preparing the viscose of lower cellulose content.

(3) From the results of the experiments on the relations between joint strengt and γ-value of viscose, and the conditions of preparing viscose, it may be probabl that γ-value is from 65 to 75 under the optimum conditions for the preparation o viscose.

189. The ripening of viscose-adhesive. IV. After sulphiding treatment. Tomo ichiro NAKATSUKA (Journ. Jap. Forestry Soc. **35**, 16-18, 1953)

The influence of after-sulphiding treatment on the properties of viscose adhesiv was studied. The decreasings of salt point, γ-value, the amount of combined alkali and joint strength take place during the ripening of viscose. These values are in creased by atfer-sulphiding treatment, with the exception of joint strength, to suc an extent that non-ripened viscose shows. It is noticeable that the joint strength o ripened viscose is not strengthened by after-sulphiding treatment.

VII. Zootechnical and Veterinary Science

Abstract Nos. 190—220

190. Serological test for a hybrid between domestic pig and wild boar. Kiyotuna ;SAKI, Kozaburo YAMAMOTO, Lyusun YOH and Tatsuo HOSODA (Jap. Journ. Zootech. :i. **24**, Suppl. 1, 7, 1953)

The blood relationship between Japanese wild boar (*Sus vittatus Japonica*), York- :re (*Sus scrofa domesticus*) and their hybrids were studied by means of precipitin action.

The results obtained are briefly summarized as follows:

The absorbed antiserum for wild boar which was exhausted completely with York- ire or with F_1 blood serum did not react with wild boar or any kinds of another ood serum.

But the reverse was not the case.

The absorbed anti-Yorkshire serum exhausted with wild boar serum, reacted ith Yorkshire or F_1 serum, and Yorkshire serum gave stronger reaction than F_1 rum.

Similarly, the absorbed anti-hybrid serum exhausted with wild boar, gave same egree of reaction with Yorkshire as F_1 serum, but the absorbed anti-hybrid serum khausted with Yorkshire serum did not react with all kinds of blood serum.

Hence, it seems to be surmisable that the domestic pig possesses a specific serum rotein in blood serum in addition to wild boar serum. And their hybrids contain l the serum components of domestic pig, but they are the middle point of both arents.

191. Serological studies on the egg laying in the fowl. (VI) On the endocrine ontrol of serum vitellin in immature chicken with special reference to their ages, exes and breeds. Kiyotsuna SASAKI, Yuichi TANABE and Tatsuo HOSODA (Jap. Journ. ootech. Sci. **24**, Suppl. 1, 7. 1953)

Serological studies on the endocrine control of serum vitellin in immature chicken ere carried out. Results obtained are summarized briefly as follows:

I. In various ages of immature chicken of White Leghorn the minimal and ptimal doses of stillbestrol which induced occurrence of serum vitellin were in- estigated.

(1) On twenty and forty days' chicken no significant difference was observed etween males and females.

(2) On sixty and ninety days chicken males required much more doses of the strogen than females.

(3) No difference was observed in point of necessary dosage for producing erum vitellin between chicken of forty, sixty and ninety days' age.

(4) Generally, necessary dosage—body weight ratio became smaller with the icrease of ages.

II. The responses of forty days chicken of broody breeds—Nagoya—might be

slightly delayed in comparison with those of non-broody breed as White Leghorn with the treatment of same daily dosage of the estrogen.

III. When thyroxin and stillbestrol were injected simultaneously to twenty days' chicken no increase in serum vitellin was produced.

IV. Occurrence of serum vitellin which had already been induced by the injection of the estrogen diminished and disappeared from their sera by the simultaneous injection of the estrogen and thyroxine.

192. On the abnormal mitosis in the testis of domestic duck. Shiichi NISHIDA (Jap. Journ. Zootech. Sci. **23**, 186-188, 1952)

It has been reported previously that the seasonal changes of testis weight are seen in domestic duck. During the period of study upon the seasonal change of spermatogenesis in domestic duck, it was found that several kinds of abnormal mitotic figures and binucleate giant cells appeared in the testes in March, April and in October, and in such materials the spermatocytes were rarely found in the tubules. Mitotic abnormalities, such as chromosome bridge, lagging chromosomes and tripolar division, were found in this study.

SNEIDER ('40) found the abnormal mitotic figures in the ovary of immature rat and cat, DEMPSEY ('39) reported the multipolar divisions and other mitotic abnormalities in the LH-administered mature guinea pig ovary, and NAKAMURA and MAKINO ('50) observed more than ten kinds of mitotic abnormalities in the experimental cryptorchid testis of ablino rat. Since the age of domestic duck used in this study are unknown, we cannot determine whether the above mentioned mitotic abnormalities are due to the age (SNEIDER) or to the hormonal unbalance (DEMPSEY), or to the degenerative stage (NAKAMURA and MAKINO). The origin of bi-nucleate giant cell seems likely to be fusion of two germ cells or nuclear division without cell division.

193. The cytoplasmic basophilia (ribo-nucleic acid) of the X-zone of the mouse adrenal gland. Koshi MOCHIZUKI and Shiichi NISHIDA (Jap. Journ. Zootech. Sci. **23**, 126-130, 1952)

By various staining methods combined with specific enzymatic digestion, the cytoplasmic basophilia (ribo-nucleic acid) of the cells of the X-zone was studied in specimens of adrenal obtained from immature and mature normal mice in a group containing both sexes and various ages, and also castrated males.

The results obtained are as follows.

(1) The cytoplasm of the cells of the X-zone exhibits basophilia after staining with basic dyes, and this cytoplasmic basophilia is interpreted as indication of ribonucleic acid, since it is abolished by treatment with ribonuclease and since its stainability in buffered solutions of dye is similar to that of other regions known to contain ribonucleic acid.

(2) The cytoplasmic basophilia of the cells of this zone showed various forms from fine granule to large rod-shaped granule, and many distribution types are found in the cells of this zone and there seems to exist some correlation between this fact and the sex and age of the mice.

(3) The deposition of the hyaline eosinophilic colloid bodies was found in the X-zone of young female, male, and castrated male mice, and some of these bodies

contained ribonucleic acid.

(4) Though many mitosis occurred throughout this zone signs of cell degeneration were rare.

(5) The facts described above, and from this point of view of the great importance of ribonucleic acid in the function of the cell (enzyme formation, protein synthesis, secretion, etc.), we are inclined to think that the X-zone may exert zone function seem to indicate that ribonucleic acid plays an important role in the function.

194. Sexual difference in the mammary development of the immature mouse. 1. Normal development and castration effect. Akira YOKOYAMA and Yoichi SYODA (Jap. Journ. Zootech. Sci. **23**, 165, 1953)

Normal development of the mammary gland in the immature male and female mice and the effect of castration on it were studied. Kasukabe strain mice, from 5 to 40 days of age, were used at 4 days' interval. Castration were carried out at 20 days of age and sacrificed at 35 and 40 days of age.

Results obtained here are summarized briefly as follows:

1. The duct system of the mammary gland of female mice grew gradually by the 25 days of age, an end-bud was not found until 25 days stage. Very rapid development of the duct system occurred during the 25 and 35 days of age and "Index of the Mammary Duct Area" of the 30 days of age were approximately eight times as large as that of the 25 days.

While, in the males, the mammary ducts remained in rudimentary state, consisting of a short main duct with a few rudimentary branches throughout the all stages examined.

Significant differences in "Index of Mammary Duct Area" were found between females and males at 5 days of age already.

2. After the castration, development of the mammary ducts of the female ceased to grow and remained in the stage of at 20 days. In the male, however, castration did not affect the mammary duct state.

3. Accessory genital organs of castrated males and females were significantly smaller than those of the normal.

From ovary and testes even in such a immature stages, and rapid development of female mammary gland occurred at 25 days stage is mainly due to the ovarian hormone (estrogen).

Further studies on such differences between male and female mammary gland development are currently in progress.

195. Sexual difference in the mammary development of the immature mouse. 4. Effect of the ovarian grafts and estrogen administration. Akira YOKOKAWA and Katsuhide TANAKA (Jap. Journ. Zootech. Sci. **24**, Suppl. 1, 14, 1953)

Ovaries were grafted subcutaneously to the Kasukabe strain non-castrate and castrate male mice at 15 days of age. And the animals were sacrificed at 25 and 35 days of age.

On the other hand, animals were castrated at 19 days of age and treated with estrogen (DESS) from the day after operation to 33 days of age once every three days. In this experiment, the total estrogen doses used were 2.5, 1.25 and 0.3 γ. These animals were sacrificed at 35 days of age.

The mammary growth obtained was compared by "Index of Mammary Duct Area". Results obtained were summarized as follows:

(1) The mammary glands of the castrate male mice bearing ovarian grafts at 35 days of age (20 days after implantation) showed remarkable extent of growth similar to that of the normal females at the corresponding age. While, in the group of non-castrate, the growth obtained was less than that of the castrate males.

(2) No significant difference in the "Index of Mammary Duct Area" was found in the cases of different dosage of Dess used in this study, but, as far as the same dosage level used, the extent of mammary growth of castrate female mice was found larger than that of males and castrate males.

(3) The mammary glands of castrate male mice bearing ovarian grafts showed more similar extent of duct growth to normal female than that of estrogen treated males and castrate males.

196. Studies on the anterior lobe of pituitary in the intersexual goats. Motoo Naito (Jap. Journ. Zootech. Sci. **23**, 56, 1952 and Zeitschr. f. Tierzüchtung und Züchtungsbiologie **61**, 201, 1953)

The anterior pituitary (AP) at the time of immaturity and maturity of genetically intersexual goats was studied cytohistologically, physiologically and histochemically in comparison with normal males and females, and castrates.

(1) No significant difference was observed between the sexes in the index of gland weight to body weight.

(2) In the cytological study of the AP, it was found that every type of secretory cell —α, β and γ—of the intersexes was larger in size than those of the normal sexes. The frequency of the α cells were similar in the several groups studied. However, the frequency of β cells in the intersexes was far lower than those of normal sexes or of castrates. The frequency of chromophilic cells in the normal sexes seemed to increase with growth until sexual muturity was reached, but this was not the case with the intersexes. The differences in the frequency of these cells in the intersexual types were not remarkable. The frequency of the β cells in the castrates was the highest of all. In mature intersexes and castrates most of the chromophilic cells were in the phase of storage.

(3) The specific gigantic cells rich in granules appeared in certain lobules of the glands of intersexes at the time of sexual maturity. They were not found in immature intersexes. These specific cells were found to be greater in size and frequency in the medial type of intersexes which had the most abnormal gonads. These seemed to originate from β cells with similar cytological aspects and showed no change in size and frequency with age after sexual maturity. In the castrates, neither "castration cells" nor related specific cells were found.

(4) The gonad tropic potency of the AP (FSH and LH) was assayed separately in each sex with infantile rats used as the recipients. In FSH the potency of the AP of the castrates was the highest and followed by that of the intersexes. In intersexes, the medial type which had the more abnormal gonads showed the higher potency of the AP of the female type of intersex was as low as that of the normal female. The potency of the AP of the male was the lowest of all mature goats. Further, the AP of immature medial intersexes showed only slight follicle stimulating action.

In regard to LH, the results obtained in the various sexes were similar to the former assays, but group differences were not so large as in the former assay with the dosage administered.

(5) In both histochemical reactions of silver and alkaline phosphatase, the intensity of the stain generally varied with the cell types; it was the most intense in the α cells, weaker in the β cells, and the weakest in the γ cells. In the specific β cells of the intersexes it was as weak as in γ cells.

The differences between sexes were more distinct in the latter reaction. Those of the normal sexes were very strong, but those of the intersexes were as weak as those of the castrates. The reaction in the immature intersex was as intense as those in the immature normal sexes, and rather stronger than those of the mature intersexes. These histochemical results in the mature animals seem to suggest that the activity of the cells in the AP of both intersex and castrate was weaker than that of the normal sexes.

(6) The cause of these abnormalities in the AP of intersexual goats is unknown, but it may be probable that genetical defect in transforming of β cells from γ cells and decrease of degranulation activity of β cells induced by disfunction of gonads may play roles.

197. I. Studies on the growth of isolated epididymis in the various conditions. II. Physiological significance of the local effect of testicular androgen on the growth of epididymis. Yoshisuke SUZUKI, Yasuo TOSHIMORI and Noboru YOTSUYANAGI (Jap. Journ. Veter. Sci. **14**, 484-485, 1952)

Surveying the colony of male rats observed, it was found that the accessory sexual organs, such as seminal vesicles and prostate glands, begin their rapid growth at about the 60th day of age, whereas, in the case of epididymis, it takes place at the 40-50th day of age, prior to that of other accessories.

In order to clarify the question why the epididymal growth starts earlier than the significant growth of accessories in the rat, the weight increase of epididymides under several conditions was investigated with the maturation progress.

The results are summarized as follows:

(1) The influence of the content from testicular secretions (sperm or other secretions) upon the weight increase of epididymis was not so significant before the 60th day of age.

(2) In the unilateral orchiectomized rat, the growth of the isolated epididymis which is located at the testis predominated significantly over that of the epididymis at orchiectomized side. The growth pattern of the latter almost fitted with that of seminal vesicles.

(3) The cause of this difference was ascertained to be the effect of the local action of testicular androgen, and not to be the outcome of the disturbance of blood supply or the abnormality of the testis etc. That is, the testicular androgen exerts its action efficiently on the isolated epididymis which is located nearly at testicle through the tunica albuginae, while the opposite epididymis received androgen supplied by the blood circulation only.

(4) The unilateral intratesticular implantation of a small dosage of desicated anterior pituitaries to immature rats, caused a significant growth of epididymis and deferent duct at the implanted testis side during the four days period, but those of

intact testis side and seminal vesicles were far less stimulated.

The local action of testicular androgen to some target organs which is located closely at testicle may take place in the physiological state, and this may account for the fact that the descent of testis, the epididymal growth, and the spermiogenesis take place before the significant growth of the sexual accessories proper such as seminal vesicles and prostate glands.

198. The hormonal treatment of the ovarian dysfunction in cows and mares. Tôsirô HOSI, Makoto YAMAUCHI, Jiroku NISHIKAWA and Tsuyoshi FUJII (Journ. Jap. Vet. Med. Ass. **5**, 321-326, 1952)

(1) Preparing an oily solution of chorionic gonadotrophin, the single injection was examined as a method of the application of gonadotrophin to the treatment of the ovarian hypofunction in cows and mares. The oily solution of gonadotrophin was prepared by homogenation of the aqueous solution of gonadotrophin with the sulfonated soy-bean oil. In the infant mouse test, this oily preparation was effective more than 3 times than the aqueous solution by the single injecting application.

For the treatment, 1520 MU or 2500 MU of this oily preparation were injected intramuscularly in mares and 1250 MU or 2000 MU in cows. In 38 out of 82 treated mares (46.3%), the heat appeared and the follicular development was observed by the rectal palpation within 15 days after the injection, and 29 out of 38 inseminated animals (76.3%) were pregnant. In 6 out of 11 cows (54.5%) treated with 2000 MU, the ovulation was observed within 10 days after the injection, and 4 out of 5 inseminated animals were pregnant, while, in case treated with 1250 MU, the result was not favorable.

(2) The cows which had the ovarian follicle cyst, were treated with the single injection of the gonadotrophin of the aqueous or of the oily solution. With small dosis less than 5000 MU, the favorable results were not obtained, while with high dosis as much as 10000 MU, the excellent results were obtained with either the aqueous solution or the oily one. In all cases received 10000 MU, the nymphomaniac symptom subsided within 3 days after the injection and all cystic follicles were luteinized with or without rupture within 5 days. Then the formed corpus luteum regressed progressively, and in some cases newly developing follicles became cystic again, but in 8 cases out of 12 treated cows (66%) the normal follicle developed and ovulated. Five out of 7 inseminated animals were pregnant.

199. Influence of the lactation on the gonadotropic activity of pituitary body. Tomonori IMAMICHI and Tôsirô HOSI (Jap. Journ. Veter. Sci. **14**, 483-484, 1952)

If the lactation period is prolonged by an uninterrupted suckling with adequate numbers of young, the estrous cycle ceases for a considerable period. This suppression of estrous cycle seems to be produced indirectly, through the anterior pituitary, by the suckling stimuli given to nipples. Concerning this problem, the pituitary gonadotropic activity in the lactating period was studied by the parabiosis of the lactating rat with the immature female.

Employing Wistar strain rats, immature females of 25 days of age were united with adult females of five different types; that is—castrated lactating mother, intact lactating mother, castrated mother without nursing, normal virgin and castrated virgin. The castration was done at the same time of the parabiotic union. The

lactating mother rat was united on the 7th day after parturition and nursed 6 sucklings uniformly. To prolong the lactation period of the parabiotic mother, sucklings were timely renewed. In case of the castrated mother without nursing, her sucklings were removed at the time of parabiotic operation.

(1) In all immature parabiants of the immature—adult castrated union (4 cases) and of the immature—castrated mother without nursing union (2 cases), the vaginal orifice opened and estrous smear appeared within from 8 to 11 days after the operation. The development of luteal tissue was observed in their ovaries, and their ovaries and uterus respectively weighed 5.8 and 6.4 times heavier than those of the immature littermate controls. The increase of the pituitary gonadotropic activity after castration was quite significantly observed.

(2) In 7 out of 8 immature animals united with the castrated lactating mother rats, vaginal estrous smear did not appear throughout 13∼27 days of the united period. As exception, one immature rat united with the mother who had significantly undergrown sucklings, showed estrous smear. In this case, milk secretion of the mother parabiant seemed to be quite scanty. In all immature parabiants of this group, none of corpus luteum was found, and their ovarian and uterine weight were not significantly different from those of the controls (0.8 and 1.6 times of control's ovaries and uterus). The pituitary gonadotropic activity of the castrated lactating mother was found to be low level.

(3) In the immature parabiants of the immature—intact lactating mother union (2 cases) and the immature—normal adult virgin union (3 cases), no significant differences were found comparing the control animals during 15 days of observation period.

From above results, the post-castration increase of pituitary gonadotropic activity may be suppressed by the effect of lactation.

200. On the respiration of horses, especially alteration of its frequency during their trotting. Hiroshi SAWAZAKI, Noriji TABA and Takayoshi MIURA (Jap. Journ. Zootech. Sci. **23**, 183-185, 1953)

The author studied on the respiration of horses during their trotting using five riding horses. Besides respiration-frequencies and steps per minute, recovery of respiration-frequency, pulse and body-temperature were observed. The results were as follows:

(1) Respiration-frequencies during exercise turned into the steady states, as described in the previous paper.

(2) Steady states' values were in proportion to the velocity of trotting.

(3) Influences of the total intensity of labour seemed to appear in the recovery of respiration-frequency and pulse.

(4) A certain relation existed between the pace and the respiration-frequency.

201. Studies on the shape of articular surface. III. About the geometrical curve, resembling the sawed articular surface of the hind leg. Shin-ichi NOMURA (Jap. Journ. Veter. Sci. **14**, 276-288, 1952)

Through a general survey on the shape and motion of the articular surface of the hind leg, I have arrived at the same computation as in regard to the foreleg. That is, the most of the joints of the hind leg are a lower pair with a surface contact,

being composed of simple stretching and bending. Analysis of the shape on a sagittal sawed plane, is a fundamental examination to determine the manner of their movement. Hence I have examined the hind leg in the same way as I examined the foreleg, and obtained the following results:

(1) The locus of centres of curvature and fluctuation of the radius of curvature show that the sagittal curve on the articulation will be nearly a 2nd degree geometrical curve, i.e. circle, parabola or ellipse.

(2) With a few exceptions, namely knee-joint etc., the curve in the hind is more nearer to circle than in the fore extremities.

(3) The calculated geometrical curves approximate sufficiently the original, especially most of the convex heads and trochleas deviated just slightly from the circle.

(4) It is also characteristic of the concave cavities that their shape tends alike rather to the parabola, and the radius of their approximate circles are always greater than those of their opposite convex elements.

(5) The deviation, which is due to a flattened deformation along the bone shaft, resulting from supporting the body weight, is less distinct than in the foreleg.

202. Studies on the shape of articular surface. IV. The shape of curve on the sagittal sawed surfaces of fresh joint praeparat and the extent and movement on the approximate circle. Shin-ichi NOMURA (Jap. Journ. Veter. Sci. **14**, 289-298, 1952)

The author has analysed the curves on the sawed surfaces of fresh joint praeparats of a light horse through the same methods as on the bone. After discussing of the curves the author has determined a proper, approximate circle to each of the curves and computated analytical-geometrically its fundamental formula and extent of movement, which was compared with the values of the practical measurement got from the same animal. The following results were obtained:

(1) The curves of the concave cavities resemble, for the most part, the circle rather than the parabola. It proved that the author's assumption on the bone praeparats was right, but near the border the curve appeared to be much flatter.

(2) On the convex trochleas the curves approximate the circle, and on the heads and condyles, rather the ellipse.

(3) The locus of a definite point on the moving bone-shaft of shoulder-, elbow- and hind fetlock-joint goes along a circle, because the radius of both elements are equal, and they can maintain a surface contact, in which each element slide one another. On the contrary, the radius of elements of the wrist-, pastern-, coffin- and hock-joint etc. are unequal, and the bone shaft moves along a epi- or hypocycloid with a sliding and rolling motion.

(4) The extent of the joint movement, calculated by the theoretical formula, shows complete a similarity to the physiological, but a remarkable difference in regard to the extent of muscular of band praeparats.

203. Mechanical studies on the elbow joint of horse. Shin-ichi NOMURA (Jap. Journ. Veter. Sci. **15**, 21-36, 1953)

The author has defined the elbow of the horse as a spiral joint, against the A. O. STOSS's opinion which regarded it as a typical hinge-joint, in consequence of the following mechanical discussions.

(1) The extent of the hinge-movement on the latero-medial axis of trochlea humeri is 90-105° physiologically. The bone axis bends and stretches along a simple curved surface. The functional axis of the forearm inclines to the lateral side, but its angle is below 5°. The instantaneous axis of bexion and stretching for every unit-movement shows irregular fluctuation in a narrow sphere, with the exception of the position of maximum stretching. (2) The pivot- i.e. screw-driver movement of the elbow joint is structural, that is a constrained rotation. The pivot-angles at the maximum position of flexion and stretching are greater than those of the intermediates. We can classify in the sulcus verticilli of trochlea the following characteristics, based on the direction of the pivot-movement and its regularity; that is, its direction brings about (a) irregular and insignificant in- and outward- (b) outward- following after inward- (c) mainly inward- screw-driver movement. (3) The real axes upon the Euler's theorem and Fischer's axes, which run parallel to them, vary irregularly in a rather narrow sphere, except at the beginning and end of the movement. The process of the axis, directed mainly by the sulcus verticilli runs smoothly along an arc and approximates that of the spiral. (4) The curves on the sagittal sawed plane of the trochlea of humerus and proximal joint surface of forearm approximate to the circle, and the projected curves of sulcus and crista have also the same character. We can decide consequently that the trochlea is composed of two cylinders, the lateral half of which has a smaller radius than the medial, and sulcus or crista is a spiral on a cylinder. The pitch of the screw varies between 0.7 and 1.8 cm. (5) Finally, I have examined theoretically the spiral character of the joint and was able to induce to the rationality for the abductive function of m. brachialis, which twine around the bone shaft of humerus.

204. A myographic study on the anti-gravitational muscles of dog. Shin-ichi Nomura and Tamotsu Ogata (Kagaku **22** (8), 1952)

Through observing the action current of the skeletal muscles induced by the coxial needle electrode, we proved the muscles which contract for maintaining the normal standing posture against the gravity when the dog stands. In the following table the mark (+) shows that the muscles work antigravitationally and (−) that they don't.

1	M. semispinalis cap.	+	16a	pars scapularis	+
2	M. cleidocephalicus	+	16b	pars acrominalis	+
3	M. serratus dorsalis	+	17	M. teres major	−
4	M. longissimus cap.	+++	18	M. supraspinatus	++
5	M. longissimus cerv.	+++	19	M. infraspinatus	++
6	M. longissimus dorsi	−	20	M. subscapularis	+++
7	M. iliocostalis	−	21	M. biceps brachii	++
8	M. trapezius cerv.	++	22	M. brachialis	−
9	M. trapezius thorac.	++	23	M. triceps brachii	
10	M. serratus vent.	++	23a	caput longum	−
11	M. sternocephalicus	++	23b	caput laterale	−
12	M. brachiocephalicus	++	23c	caput mediale	++
13	M. omotransversarius	+	24	M. ext. carpi rad.	−
14	M. pectoralis super.	+	25	M. ext. carpi uln.	−
15	M. pectoralis prof.	+	26	M. ext. dig. comm.	−
16	M. deltoideus		27	M. flex. carpi rad.	+++

28	M. flex. carpi uln.	+++
29	M. ext. dig. lat.	−
30	M. flex. dig. super.	+
31	M. flex. dig. prof.	
32	M. glutaeus super.	++
33	M. glutaeus medius	++
34	M. glutaeus prof.	++
35	M. tensor fasc. lat.	+
36	M. biceps femoris	−
37	M. sartorius	+
38	M. semimembranosus	−
39	M. semitendinosus	−
40	M. gracilis	++
41	Mm. adductores	
42	M. vastus lat.	+++
43	M. vastus med.	+++
44	M. rectus femoris	++
45	M. tibialis ant.	−
46	M. ext. dig. ped. long.	−
47	M. gastrocnemius	+++
48	M. fl. dig. ped. sup.	+
49	M. fl. dig. ped. prof.	

In the dog, on the whole, the manner of fixing the moval joints and girdles is notably different from the hoofed animals, because he keeps the advance potsure while he stands.

Number of the neuromusclar units of the dog is more than the hoofed animals, and thus the spike-discharge intervals fluctuate about 0.07-0.10 sec.

205. Myographic studies on the anti-gravitational muscles of horse and cattle. Toshio OKABE, Shin-ichi NOMURA and Hiroshi SAWAZAKI (Journ. Jap. Vet. Med. Asso. **6**, 121-126, 1953)

The so-called anti-gravitational muscles which work to maintain the normal standing posture, can be determined easily through the myogram induced from the skeletal muscles. Among the principal muscles in the following table, those marked by (+) contract to fix the moval joints and girdles, and to keep the posture against the gravity, when the horses and cattles stand.

		horse	cattle
1	M. long. cap.		−
2	M. longiss. cap. atl.	++	+
3	M. rect. cap. dors.		+
4	M. splenius	++	−
5	M. serratus dors.		
	pars cerv.	+++	++
	pars thor.	+++	++
6	M. rhomboideus		+
	pars cerv.	++	
	pars thor.	++	
7	M. sternohyoideus		−
8	M. sternocephalicus		−
9	M. brachiocephalicus	+++	+
10	M. spinalis et semispinalis	−	−
11	M. sacrospinalis	−	−
12	M. latiss. dorsi	±	−
13	M. deltoideus	±	++
14	M. trapezius		
	pars cerv.	+	++
	pars thor.	++	++
15	M. pectoralis prof.	+++	+++
16	M. pectoralis super.	++	++
17	M. subscapularis	++	+
18	M. supraspinatus	+++	+++
19	M. infraspinatus	++	++
20	M. teres major	−	−
21	M. teres minor	+	+
22	M. triceps brachii		
	caput long.	−	−
	caput med.	++	+++
	caput lat.	++	+++
23	M. anconaeus min.	++	
24	M. biceps brachii	±	−
25	M. brachialis	±	−
26	M. ext. carp. rad.	+	+++
27	M. ext. carp. uln.	+	++
28	M. ext. dig. comm.	−	−
29	M. ext. dig. lat.	++	+
30	M. flex. carp. rad.	+++	++
31	M. flex. carp. uln.	+++	++
32	M. flex. dig. prof. et supper.	++	+
33	M. rect. abdom.	−	−
34	M. obliq. abdom.	++	+

35	M. glutaeus super.	+	+	46	Mm. adductores	−	−
36	M. glutaeus med.	+	⧻	48	M. tibialis ant.	−	−
37	M. glutaeus prof.	+	⧻	49	M. gastrocnemius	⧻	⧻
38	M. biceps femoris	−	−	50	M. peroneus tertius		−
39	M. semimembranosus	−	−	51	M. tensor fasc. lat.	+	+
40	M. semitendinosus	−	−	52	M. ext. dig. ped. long.	−	−
41	M. rectus femoris	⧻	⧺	53	M. ext. dig. ped. lat.	±	−
42	M. vastus lat.	⧻	⧻	54	M. flex. dig. ped. super.	+	+
43	M. vastus med.	⧻	⧻	55	M. flex. dig. ped. prof.	+	

With a few exception of supporting the head and neck, for which the muscles around them contract, the action of the chief muscles that is characteristic of the horses and cattles is characteristic of the other hoofed animals as a whole. The fixation at the upper parts of the body is brought about primarily through the contraction of muscles bilaterally, while at the lower parts unilaterally with assistance of the extended joints.

Number of the N.M.U. is considerably less than that of the dog, thus the spike-discharge intervals vary between 0.10 and 0.15 sec.

206. Requirements for origination and conduction of the peristalsis. Yoshio Okubo and Iwao Yanagiya (Folia Pharmacol. Japon **47**, 189-190, 1952)

It was demonstrated in our previous communication (this records No. 11, p. 90) that the distension of the intestinal wall is essential for occurrence of the peristalsis.

Further studies have been done to make the finding more precise (1) Studies on role of the movement of either of both the muscular layers. (2) Significance of the distension wave prior to the peristalsis. (3) Role of the intestinal ganglion for the intestine movement.

Exp. 1. Increasing the pressure in intestinal lumen by the Trendelenburg's method, the volume of the intestinal strips increased, but its length shortened.

a) Prevented the distension of the circular muscle layer. Prevented the distension of the intestinal strip by covering with a glass tube, the constriction did not occur, namely the peristalsis disappeared.

b) Prevented the shortening the longitudinal muscle layer. Fixed both ends of the intestinal strip to avoid the longitudinal shortening in the Trendelenburg's method, contraction waves of the circular muscle layer, namely the peristalsis existed. It was found that an essential phenomenon for origination of the peristalsis is not the longitudinal shortening but the distension of the circular muscle layer.

Exp. 2. To clarify the significance of the distension prior to the peristalsis, it was determined whether the conduction occurred or not on the intestinal strip inserted into a just fitting glass tube with a globular expansion at the site of which the strip expanded locally and peristalsis originated there when pressured.

Results showed the peristalsis originated at the oral part of the expansion and conducted until the anal end of the expanding part and disappeared there. These results support a view that the proceeding distension is essential for the peristalsis.

Exp. 3. The peristalsis was arrested by adequate amount of hexamethonium which caused paralysis of the intestinal ganglion (nicotine test). It is showed that the intestinal ganglion plays the essential role in keeping the occurrence of the peristalsis.

207. Acetylcholine production in passively extended wall of the intestine. Nobuyuki CHUJYO (Amer. Journ. Physiol. **170**, 3, 1952)

In view of the effects of acetylcholine (ACh) on the intestinal movements, it may be probable that some relationships exist between the extension and the production of ACh in the wall of the intestine. In this paper the author examined the ACh content in the wall of the intestine and in the solution in which the intestinal segments were placed, both in extended and freely contracted states.

The guinea pig small intestine was used. Forty minutes after putting the segments into the saline solutions, the ACh content of the solution was determined. In order to determine the ACh content in the wall of the intestine, the segments were frozen with dry ice and ground with a mixture of sand, eserine solution and HCl. The extract was used for ACh determination. The ACh assay was carried out on the frog rectus muscle sufficiently eserinized in $NaHCO_3$-free RINGER's solutions containing eserine salicylate.

Results: (1) ACh content in the sol.

The ACh content in the sol., in which the extended segment was immersed, comes within the range of $2.1\text{-}3.5 \times 10^{-8}$ GM/cc except 2 cases of "trace". In the solution of the non-extended segment, the author failed to detect in the most of cases.

(2) Nature of the solution.

The solution which was active on the rectus muscle becomes inactive by alkalinization, with NaOH, or boiling. With these properties and the others, it can be surmised that the substance which is found in the solution in which were kept the intestinal segments, and which cause the contraction of the rectus muscle may be ACh.

The author found by further experiments that no substance which augments the action of ACh appears in the solution.

(3) ACh content in the wall of the intestine.

The extended wall of the intestine contains obviously larger amount of ACh than the non-extended, except in one case. The ACh content in the extended intestinal wall comes within the range of $6\sim20\,\mu$ GM/GM. of tissue.

208. Observations on the evacuation of the cecal contents in the chickens. Miyoshi IKEDA (Folia Pharmacol. Japon **49**, 54, 1953)

It is well known that the intestinal contents of the chickens are usually discharged in the two different forms, one passing the ceca and the other without passing it, and the former is called "cecal dropping", the latter "intestinal dropping", both of which are easily distinguishable with their appearances or characters. Knowledge on the mechanism of the evacuation of the cecal dropping is less than that of the intestinal dropping. Following experiments were attempted to make it clear.

A. Observation of the passing of the intestinal contents. According to a method of detecting the bone-charcoal marked food, it was observed that the intestinal contents reached near the opening of the ceca at an hour after the food administration, the end of the rectum after an hour and a half and appeared slightly in the droppings, increasing gradually thereafter, and disappeared after about 8 hours. On the other hand, a part of the intestinal contents entered into the ceca at about 2 hours after feeding, then more markedly at 3 hours and the charcoal became undetectable in the cecal droppings after 2-3 days.

B. Observations on the evacuation of the cecal contents.

(1) The case of the chickens with the normal feeding (normal condition).

a) The 3-4 cecal droppings per bird per day were discharged but not at all discharged at night. from 7-8 p.m. to 5-6 a.m. next morning, while chickens were asleep.

b) While awakened in the daytime, there appeared two peaks in the evacuation of the cecal droppings, morning peak (6-8 a.m.) and afternoon peak (3-6 p.m.). The latter was greater than the former.

c) In contrast with the above, the intestinal droppings were discharged through day and night without showing the usual peaks as above described.

(2) The case of the chickens with the control of the natural light.

The chickens were placed in a dark room, where the lights had been avoided, in the evening and left still as they were, up to 10-11 a.m. next morning. As soon as the lights were turned on abruptly at 10-11 a.m., these chickens became active and all had discharged the cecal dropping rapidly within an hour. Thereafter the evacuation of these chickens showed the usual course almost identical with that of the chickens kept under natural light, except that the morning peak was earlier and much more abruptly.

(3) The case of the chickens whose movement was controlled.

Effects of the condition that the movement of the chickens were controlled, were observed. Under such conditions, there was no cecal droppings, although the intestinal ones were discharged. As soon as the chickens were unfastened and became free, the cecal droppings were discharged abruptly and then the usual evacuation followed.

From the above results, it seemed that there was a correlation between the movement of the chickens and the cecal evacuation.

209. Cesarean section in cattle. Masaaki HATAYA, Kazuya USUI, Hikozo ICHIKI, Tetsuo FUJITA and Shigekazu MOTOYOSHI (Journ. Vet. Med. **100**, 7-8, 1952)

It has been reported that various difficulties encountered during the period from pre- to postparturient time in animals are frequently resorted to the surgical procedures. The operation of cesarean section has been indicated in those cases that there is disturbances of the genital canal, particularly due to deformities of the bony pelvis and non-dilation of the cervix, abnormally large fetus, emphysematous fetus, malposition of the fetus, uterine hernia etc.

In 1952, the writer was fortunate in performing the operation in three cases of cattle. Those were the cases of an abnormally large fetus due to delayed parturition, a mummified fetus and a uterine torsion immediately before calving, respectively.

The operations were performed, under local infiltration anesthesia, on the right flank of the cows which held themselves on foot.

Case (1) Japanese brown breed of cattle. 4 years old. Operated on 6, 5, '52.

Animal examined in the evening of 5, 5, '52, when the attempts to effect calving such as administrations of posterior pituitary extracts, stilbesterol and quinine, instillation of vagina and uterus with large amounts of lukewarm water were all failed to dilate the cervix and expulse the fetus. The parturition was delayed over 45 days after the scheduled date. Although not in very good bodily condition, the animal was still standing and eating some roughage. It was decided to carry out cesarean section and the operation was immediately performed. A very large, dead bull calf was delivered. Fetal membranes left in situ. Animal was found to be in

excellent condition in a week, but suture abscesses were left.

Case (2) Friesian-Holstein, 4 years old. Operated on 15, 5, '52.

Animal, on the ninth month of gestation, held a mummified fetus which had been alive in so far as fifth month and died later by unknown reason. The owner sought surgical aid inasmuch as the cervix of the uterus had not responded to the administrations of several doses of posterior pituitary extracts and stilbesterol.

Cesarean section was performed and the mummified fetus, situated in the right horn of the uterus, was removed. The wounds of the uterus and of the abdomen recovered uneventfully.

Case (3) Friesian-Holstein, 5 years old. Operated on 10, 6, '52.

Animal had been attempting to calve since the night before in which calving had been just scheduled. Next morning, a practitioner had found out the twisted uterus and asked us for an aid because of non-dilation of the cervix. Cesarean section was carried out in the afternoon. A fetus, situated in the left horn of the uterus, was lying on the opposite side of the abdominal cavity and easily accessible through the incision on the right flank. A female calf was delivered in a state of suspended animation and efforts to stimulate breathing were not followed by normal respiration. The fetal membranes were easily removed. The dam recovered uneventfully.

It is far better to resort to the operation of cesarean section prior to the animal falls into a desperate condition, when the maternal passage is for some reason unable to accommodate and discharge the fetus. The writer considers that the day of extensive embryotomy has gone forever since the operation can be carried out in one to two hours with far less strain on both patient and operator, and the development of sulfonamides and antibiotics has also remarkably diminished the danger of septic complications to the operation even in large animals.

210. Anesthetic effects of chloral hydrate on chickens. Hakaru KUNISAKI, Norimoto URAKAWA and Yosio OKUBO (Jap. Journ. Vet. Med. Asso. **6**, 167, 1953)

Studies on anesthetic effects of chloral hydrate on chickens have been performed as a part of the systematic work conducted by a research committee on the anesthetics for domestic animals.

(1) The tolerant dose of chloral hydrate seems to be about 250 mg/kg. Doses of 300 mg/kg of chloral hydrate caused 4 in 10 chickens.

(2) Anesthetic effects.

The chicken laid down at 15 minutes after the subcutaneous injection of 3 per cent chloral hydrate (200 mg/kg). Thirty minutes after, the pain reflex of the skin with the hooked pincette disappeared and the response of the cockscomb became slow. But the muscular relaxation of the leg did not appear. This condition corresponding to a stage of the medium anesthesia in the WRIGHT's criterion continued for 15 minutes.

Then the chicken awakened gradually. After 8 hours, the chicken crouched and 10 hours, stood up. They showed neither hyper-sensation nor emaciation at awakening.

(3) General symptons.

At thirty minutes after the injection the body temperature decreased distinctly, the respiration became shallow, also the pulse decreased, the cardiac sound weakened, and the peristaltic sound of the intestine disappeared. Four to six hours thereafter

the respiration, the pulse, the peristaltic sound recovered, but only the recovery of the temperature was in need of duration of 10 hours.

(4) Side action.

The blood pressure decreased continuously, the blood sugar dchanged scarcely, and the blood rest-nitrogen did not decrease but a little fall 30 minutes after.

211. The anesthetic effects of Ravonal (thiopental sodium) on the dog. (Correlation betdeen dose and duration in the Ravonal intravenous anesthesia.) Masaaki HATAYA, Kazuya USUI and Tetsuo FUJITA (Jap. Journ. Vet. Sci. 14, 478, 1952)

Thiopental sodium is used only in the cases of good anesthetic risks and for operative procedures in which anesthesia for from five to twenty minutes is required. It is not recommended in major operations calling for prolonged anesthesia.

It is reported that the dosage of thiopental sodium can not be accurately judged by weight, age, sex, metabolic state etc., and it must be adjusted to each individual patient inasmuch as susceptibility of patients for the drug is widely divergent.

As to the dosage of thiopental sodium when it is administered to the dog, it is described that the anesthetic dose belongs generally to a considerably wide range such as reported 1/8-1/6 gr/lb by SWEEBE & KHUEN (1936), 15-20 mg/kg by PRATT et al (1936), 20-35 mg/kg by WRIGHT (1937), 1/9-1/5 gr/lb by SCHNELL (1947). The present experiments were designed to study this problem.

The 2.5 per cent aqueous solution of Ravonal (Tanabe Pharm. Co.) was administered intravenously with a constant rate, 10 seconds per each cc, to 68 adult mongrel dogs, and the correlation between dosage and duration of anesthesia was determined.

The results obtained were as follows:

(1) There was a highly intimate correlation between dosage per kg of body weight and duration of surgical anesthesia in the case of intravenous Ravonal anesthesia in the dog. The coefficient was 0.66.

(2) The correlation was not of a straight line and was indicated by a formula $Y = 10.38 \times X^{0.335}$ (Y: mean dosage, mg/kg B.W., X: mean duration of surgical anesthesia, minutes).

The administration of dosage from 10 to 20 mg per kg of body weight obtained a little too short duration of anesthesia for surgical procedures and that of 30 mg per kg of body weight or more caused not infrequently to happen unfavorable side- and after-effects.

It was concluded that the dosage of 25 mg per kg of body weight intravenously was most profitable for the anesthesia of short duration in the dog and Ravonal exhibited the most favorable effects only when it was employed as an anesthetic of short duration.

212. Comparative studies on the anticoccidial effects of various drugs on experimental coccidiosis (*E. tenella*) in chickens. (I) On the anticoccidial effects of various drugs. Yoshio OHKUBO and Miyoshi IKEDA (Journ. Jap. Vet. Med. Ass. 14, 358, 1952)

(1) The present report deals with the study of comparison on the anticoccidial effects of various drugs and the study of determination of therapeutic and prophylactic doses of the effective drugs. The "therapeutic" dose is the minimal effective dose which, if administered at certain stages of chicken-coccidiosis, prevents completely its clinical symptoms and the "prophylactic" dose is that which is not sufficient to prevent completely, but may be effectively used in long-term continuous medication.

The therapeutic and prophylactic doses of the drugs are respectively as follows: Furacin, 0.016%, 0.01%; Bisphenol, 0.4-0.5%, 0.25-0.3%; Nitrophenide, 0.05-0.06%, 0.02-0.025%; 4-chlorophenyl arsonic acid, 0.015%, 0.005-0.008%; 3-nitro-4-hydroxyphenyl arsonic acid, 0.025%, 0.0125%; Sulfamerazine, 0.3%, 0.2%; Sulfaquinoxaline, 0.05%, 0.025-0.03%.

(2) Studies on the treatment started on different stages after infection of *E. tenella* to chickens revealed as follows: Bisphenol and 4-chlorophenyl arsonic acid are fully effective if given before infection, but immediately or later after infection, their therapeutic effects reduced markedly. It seems that these drugs may inhibit the early developmental stage of *E. tenella*. The other drugs prevent the mortality from coccidiosis if given no later than 2-3 days after infection.

(3) An attempt was performed to ascertain the effects of the treatment on the sporulation of oocysts discharged by infected chickens which were treated with effective dose of the drugs for 3 days from 7th after infection. The following results were obtained: The rate of sporulation of oocysts varies with the drugs, and Sulfamerazine and Nitrophenide inhibit more markedly the sporulation of oocysts, as compared with those of the other drugs, namely, percentages of sporulation of oocysts were 16% in Sufamerazine, 23% in Nitrophenide and 71% in untreated control.

213. Comparative studies on the anticoccidial effects of various drugs on experimental coccidiosis (*E. tenella*) in chickens. (II) On the toxicity of drugs. Yoshio OHKUBO, Miyoshi IKEDA and Norimoto URAKAWA (Journ. Jap. Vet. Med. Ass. **14**, 359, 1952)

(1) As it had been observed that the lethal dose of Furacin to chickens must vary according to the age of the chicken, chickens used in the experiments were about 80-100 grams, and of male White Leghorn born on the same day.

The median lethal doses of drugs were found respectively to be as follows: Furacin, 275 mg/kg; Nitrophenide, 1.87 g/kg; Sulfamerazine, 3.8 g/kg; 4-chlorophenyl arsonic acid, 9.2 mg/kg. LD_{50} in Bisphenol was too large to give a single dose.

(2) The therapeutic (prophylactic) index of drugs, therapeutic (prophylactic) daily dose $/LD_{50} \times 100$, increased in the order of Nitrophenide, Furacin, Sulfamerazine and 4-chlorophenyl arsonic acid.

(3) Effects on the weight gains of chickens received continuously the prophylactic doses of the therapeutic doses of the drugs were observed over a 25 days period. Continuous medication of the prophylactic doses of drugs had no appreciable retarding effects, as compared with those of untreated controls, but growth-retarding effect resulted from medicated diets containing the therapeutic dose, 0.022%, of Furacin, though in the other drugs the weight gains did not differ from those of untreated controls.

(4) The acute toxicity of drugs in chickens receiving a large single doses of drugs was observed.

The intestine, kidney and liver responsible for absorption and secretion were markedly affected, according to the microscopic examination, due to the toxic doses of drugs and particularly most affected by the arsonic compounds, although Sulfamerazine, Nitrophenide and Furacin were slight.

214. On the prophylaxis and treatment of lumbar paralysis of sheep and goat.

Shin OKOSHI (Animal Husbandry **5**, 137-140, 1951)

So-called Lumber Paralysis is caused by the larvae of *Setaria digitata* which lives in the abdominal cavity of cattle and strys into the cerebro-spinal nerve of the abnormal host.

The intermediate host of *Setaria digitata* are three kinds of mosquittoes (*Aedes togoi, Armigeres obturbans* and *Anopheles hyrcanus* var. *sinensis*). This disease occurs only in a graminivorous animals as sheep, goat and horse in Japan and Korea, frequently from July until October.

There are no change of the body temperature, frequency of respirations and pulse-rate.

The main symptom is the nerve paralysis of the rear body.

In a mild case, the animal reels and in a serious case it can not stand up. Besides, there is the crook of the neck, the concussion of an eyeball and paralysis of the penis.

The period of incubation is not constant, the average period of incubation is about a month, but the shortest period is 15 days.

As a medical treatment of this disease, the intravenous injection of antimony compounds obtains the desired result to destroy *Setaria digitata.*

The prevention of Lumbar Paralysis is desirable that the very same preparation and treatment is performed, and it is a sort of an early expulsion of the worms.

215. Studies on liver function test by bromsulphalein for the antimony poisoning. Shin OKOSHI, Kiyoshi SAITO and Tatsuo NAKAHARA (Jap. Journ. Vet. Sci. **14**, 351-352, 1952)

The author have tried an experiment on the liver function by bromsulphalein for the antimony poisoning of the domestic animals.

After the infection of sodium-tartar-emetic and sodium-thiosulfate to dogs and goats, Bromsulphalein was injected. Thereafter we collected their serum after 5, 10, 30 and 45 minutes respectively.

Results were as follows:

(1) Every antimony poisoning dog showed intense positive.

(2) Every dog, died by poisoning, showed higher than 18% bromsulphalein blood level in 45 minutes value.

(3) Every dog, simultaneously injected sodium-tartar-emetic and sodium-thiosulfate, showed negative or weak positive.

(4) Every goat, injected sodium-tartar-emetic, showed negative, and could not find any disturbance of the liver function.

216. Studies on kidney function test under various influences of antimony compound and sodium-thiosulfate injection. Shin OKOSHI, Tetsu SHIMABUKURO, Yukitoshi INOUE and Manpei USUI (Jap. Journ. Vet. Sci. **14**, 459-460, 1952)

It occurs severe poisoning after injecting antimony compound to the domestic animals. So the authors tested the kidney function on this case.

The author have tried an experiment on the kidney function by Phenol-sulfonphthalain with urine and Xanthoprotein Reaction Probe with serum for the dog, injected sodium-tartar-emetic and sodium-thiosulfate.

Results were as follows:

(1) The kidney function test was positive for the pregnant dogs.

(2) The kidney function test was negative for the healthy (not pregnant) dogs.

217. Studies on the hippuric acid synthesis test under various influences of antimony compound and sodium-thiosulfate injection. Shin OKOSHI, Kiyoshi SAITO, Takeo TAKAHASHI and Toshio ISHII (Jap. Journ. Vet. Sci. **14**, 460, 1952)

Under the antimony poisoning of domestic animals, the authors have tried an experiment on the liver function by the hippuric acid synthesis test.

For the goat, after the intravenous injection of sodium-tartar-emetic and sodium-thiosulfate, which is a counter poison for antimony compound, sodium-benzoate was given orally. Thereafter the author collected the urine with the lapse of time within four hours and hippuric acid synthesis test was carried on.

(1) After the intravenous injection of sodium-tartar-emetic, the amount of synthesized hippuric acid decreased distinctly, and recovered to normal after eight to twelve days.

(2) After the intravenous injection of sodium-tartar-emetic and sodium-thiosulfate at the same time, the amount of synthesized hippuric acid decreased, but after four days it recovered.

(3) After the intravenous injection of sodium-thiosulfate, the hippuric acid synthesis test gave negative results. So the authors could find no disturbance of the liver function.

218. L'encéphalite japonaise des porcs. Yuichi OCHI (Bull. Off. Internat. Epiz. 504-517, 1953)

Though the epidemic of infectious encephalitis has been known for long years having the big outbreaks repeatedly with the interval of about 10-15 years, the entity of this disease had not been clarified until the recent prevalence of 1947-1949.

According to the investigations made during the course of the recent outbreak, it was concluded that the infectious encephalitis in horses and human beings is the same disease caused by the same agent which is quite different from any encephalitis virus reported in other countries. We named, accordingly, the virus as "Japanese Encephalitis Virus" and the disease was decided to be called the "Japanese Encephalitis in man and domestic animals".

Although the disease has been observed only in horses, it was recognized, in the recent outbreak, that not only horses but cattle, goats, pigs and other small animals are also susceptible to this disease. The disease in pigs is not only important in the practical view-point but also very interesting when considered from the scientific angle, with its high incidence followed by considerable damages on livestock industry and its peculiarity in the nature of symptoms which are very different from that of other animals.

The most characteristic symptom is abortion or still-birth in pregnant sow. Pregnant sow, though infected, remains healthy in appearance without any symptom till the abortion or still-birth which occurs after the time of expected parturition. The litter varies in size remarkably; some are small in size and mummified, some are in normal size and some are in life briefly after the birth with normal development.

Non-pregnant pigs are usually affected with the form of latent infection while

the young pigs often manifest the symptoms of encephalitis.

All the dead-borns, mother sows and infected young pigs with encephalitis symptoms show pathologically, more or less, the picture of non-purulent encephalitis as it is the case in man and horses.

Serologically, the mother sow, which underwent abortion and still-birth, and young pigs with encephalitis symptoms show positive reaction against both the neutralisation and complement fixation tests. Besides the above mentioned, there are many in the infected area which have neutralisation body in blood and it shows that there are also many pigs suffering from latent infection as it is in other species of animals.

The facts that many strains of virus have been isolated from the foetus and the affected piglet, and all of them have been identified with Jap. B encephalitis virus, prove clearly that the abortion and the still-birth in pigs are caused by the infection due to this virus.

With the artificial infection on pigs which are negative in the preliminary neutralisation test, piglets easily infected manifest fever, viremia, appearance of neutralisation anti-body, etc. In this case the symptoms of encephalitis are relatively rare except when the virus was inoculated intracerebrally, and most of them show the latent form of infection as it is observed in the natural infection. Pregnant pigs when inoculated with the virus which passed through only a few passages give abortion or still-birth just as natural infection. Thus, it is possible to reproduce artificially the disease as it was in natural.

Employing killed vaccines prepared with the virus, experiments were carried out against the artificial infection with good results. As the field trials were also very satisfactory, the vaccination is now widely in use in Japan to prevent the disease.

219. La fièvre épizootique des bovins au japon. Yuichi OCHI (Bull. Off. Internat. Epiz. 518-133, 1953)

An acute infectious disease of cattle with high fever, which is called "Cattle Influenza" by name since the beginning and prevails heavily at intervals of about 20 years, has been known for long years in Japan. In the recent outbreak (1948-1950), the epizootics prevailed so severely and the damages caused were so serious that a Research Commission on Cattle Influenza was appointed by the Japan Veterinary Medical Association bringing together the specialists concerned from various fields. After laborious studies, it was revealed that the disease is an infectious disease peculiar to cattle caused by a virus and it looks like more similar to "Ephemeral Fever" or "Three-day's Sickness" rather than Influenza. Accordingly the Commission decided to abolish the old nomenclature and call it newly "Bovine Epizootic Fever" to avoid the confusion with human influenza.

According to the record, the large prevalences of the disease were found in 1887-1891, 1906-1908 and 1929 with several hundred thousands of animals involved in each time, and a big outbreak of 1949-1950 followed them. Generally, the disease appeared in June or August, and ceased in December.

The cause of the disease is a virus, which is contained abundantly in blood at the access of fever. By the successive passage through cattle, the virulence of the virus may gradually increase and after 15-20 passages, it reaches 10^{-6}. The virus maintains its virulence for 6 months under the frozen condition.

Until now, the experiment of artificial infection on chick embryo and animals other than cattle are not successful.

The most characteristic symptom of the disease is a sudden high fever of 40-42°C., which comes in the incubation period for 2-7 days, in average 3 days, and the sudden lysis thereof after 1-3 days of continuation. During the period, the cattle lies in the corner of the stable, showing the serious symptoms such as inappetance, accelerated respiration, shivering, palpitation, etc.. . Besides those, other symptoms may be observed by individuals, in the eyes, respiratory organs, digestive organs, nervous system and so on. In the recent epizootics, laryngopharyngeal paralysis which had never been observed in the past epizootics, showed itself as a peculiar symptom seen in about 1% of affected cattle.

Among the pathological changes of the case which had the typical symptoms, interstitial emphysema of lung is most remarkable and no other important change is seen except the swelling of spleen, liver, kidney and lymphatic glands. In the case which had laryngopharyngeal paralysis, there are different lesions such as serious congestion and hemorrhage of membrane of digestive organs, degeneration of muscles of oesophage, larynx, pharynx, diaphragma, intercostal muscle, body muscle, etc..., pneumonia due to foreign body, regressive degeneration of nerve cells in central nervous system, particularly in medula oblongata.

It is believed that the propagation of the disease is exclusively caused by direct or indirect contact and that the rôle of blood sucking insect is not so important.

Generally speaking, the course of disease is mild, recovering rapidly after 1-3 days with the mortality of about 0.5%. But, in the recent epizootics, there were many cases with the symptom of laryngopharyngeal paralysis in which the prognosis is very malign with higher mortality.

As for the treatment, only the allopathic methods are in use, having no radical method for cure. In case of laryngopharyngeal paralysis it is essential to supply water and nutritions artificially. For this purpose, the use of stomach probe or trocar is recommended with good results in pouring water into rumen directly.

As for the prophylactic, crystal violet killed vaccine is now in use successfully in this country.

220. Studies on AVIAN type tuberculosis. V. The VILLEMIN type tuberculosis in rabbits. Shutaro YAMAMOTO, Kiichi ISHIDA, Kosaku FUJIWARA and Aiko SATO (Transac. Soc. Path. Jap. **41**, Edit. Reg. 291-293, 1953)

The authors performed a serial observation of the VILLEMIN type tuberculosis with 26 rabbits, which received intravenous injection with 0.001 mg. per kilo. of the Flamingo strain. Histopathological examination of various organs and supravital observation of the lesions as well as the quantitative culture of the bacilli in the organs had been performed.

The amount of the bacilli in all organs examined increased gradually until the 3rd or 4th week, and it was dominant in the liver, spleen and bone marrow, the same as in the YERSIN type tuberculosis. Then the bacilli began to decrease until the 10th week and the differences among the organs became smaller. At the end of the 15th week the bacilli again increased in number, and this time the distribution of the bacilli in the organs appeared in the reverse order; namely, they are dominant in the lung and kidney. The results obtained here are similar to that of LURIE, who

examined experimental human and bovine tuberculosis in rabbits by his quantitative culture method. Later THOMAS in his study of experimental bovine tuberculosis in rabbit concluded this transition of the lesions as "Diphasic Nature of Tuberculosis".

The histo-pathological figures change in parallel with the amount of the bacilli. Intradermal tuberculin test gave distinct positive reaction at the 6th week.

The lesions in the liver appear as small accumulation of the mononuclear cells in the sinusoids 2 weeks after inoculation. 25 days after inoculation lesions show well circumscribed miliiary tubercle-like appearance and supravital preparation of the lesions reveals round mononuclear cells with rosette of fine neutral red vacuoles. Compared with the same stage of YERSIN type, the extent of the hepatic lesions are far more limited but there is marked tendency to the tubercle formation. After that the lesions of the liver decreased in number, but from the 8th to 10th week the size of the lesions began to increase accompanied with spreading of central nercotic areas. The lesions in these stages are most commonly seen in the interstitial tissues and it is a very unique finding that there are large swollen mononuclear cells accumulated in the lymphatic vessels of GLISSON's sheath. Authors supposed these cells might have transfered from intralobular lesions through DISSE's cavity, and farther transference of these cells to the portal lymph node was considered. In fact, severe sinus-catarrh and tubercle-like accumulation of large mononuclear cells are seen in the portal lymph node. In the mesenteric lymph node of these stages the lesions had never been seen in such a degree. 20 weeks after inoculation, hepatic lesions diminished and there developed some secondary lesions.

The lung showed round interalveolar accumulations of the large mononuclear cells already after 25 days, and at the end of the 20th week there are accumulation of large swollen mononuclear cells filled in the moulds of alveoli other than interalveolar mononuclear accumulations. Lesions of the large mononuclear cells appear in the renal cortex 4 weeks after inoculation. Central necrosis appears at the 6th week and the lesions spread to the medular substance 8 weeks after inoculation. At the end of 15th week the lesions finally break into the pelvis of kidney, where numerous tubercle bacilli are seen. Accumulation of the large mononuclear cells also appears in the testicles from about 10 weeks after inoculation, which soon undergoes central necrosis.

The authors conclude that other than gross distribution of the lesions, the difference from the YERSIN type appears most distinctly in the histo-pathological figure of the liver, where marked tendency to the tubercle formation in the early stages is recognized and transference of the large mononuclear cells to the lymphatic vessels of GLISSON's sheath by way of DISSE's cavity was observed at the later stages of the disease.